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FEEDING EXPERIMENTS WITH ISOLATED FOOD-SUBSTANCES

BY

THOMAS B. OSBORNE and LAFAYETTE B. MENDEL
With the Co-operation of EDNA L. FERRY

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FEEDING EXPERIMENTS WITH ISOLATED FOOD-SUBSTANCES.

PART II.

INTRODUCTION.

In Publication 156 of the Carnegie Institution of Washington* we have discussed some of the problems of nutrition which have been raised by the newer investigations in the field of protein chemistry. The literature bearing on the feeding of isolated proteins was there reviewed in some detail, together with critical considerations of previously available experimental data. We described a plan for the study of metabolism and illustrated a method of investigation in which white rats were the experimental animals. For the details involved, our earlier paper must be consulted. A few protocols were there presented to show that the outlined mode of investigation offered a promising means for attacking certain questions in the field of nutrition.

INFLUENCE OF VARIOUS CONDITIONS ON NUTRITION OF WHITE RATS.

Numerous contingencies may arise to modify or vitiate the results of experiments in which animals are kept in cages and fed upon artificially prepared mixtures of isolated food-stuffs, quite independent of the factors inherent in the food-stuffs themselves or the combinations in which they are exhibited. Among these possibilities, the *caging* itself, involving continued restraint and limited opportunity for exercise, suggests an unfavorable environment. This factor can at length be disposed of.

Donaldson has concluded, from the best data obtainable, that "the three-year-old white rat is very old, and is justly comparable to a man of 90 years."† Rats have been kept in our cages in apparent good health and without difficulty during periods of more than 14 months—a very considerable part of the span of life in these animals (cf. Charts XXIII, XXIX, XXX).

*Feeding experiments with isolated food-substances, by Thomas B. Osborne and Lafayette B. Mendel, with the co-operation of Edna L. Ferry. 1911. Pp. 53.

†H. H. Donaldson: A comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume, New York, 1906, p. 6.

Monotony of diet has been urged as an obstacle to success where the same food mixtures are daily furnished without change over long periods of time. Very closely associated with this is the question of the *palatability* of the diet. The two factors need, however, to be distinguished. The palatability of the diet has, perhaps, been overemphasized in recent years in its bearing on the real nutritive value of foods. It applies primarily to the individual with highly organized nervous system and psychical functions. The quality found in foods which are unpalatable because they disgust or nauseate is something positive; the negative property of lack of palatability, *i. e.*, absence of stimulating taste, etc., is not necessarily a serious obstacle. In any event the palatability of the diet is difficult to determine or regulate and in attempting to control it experimentally in animals physiologists have been guided very largely by anthropomorphistic considerations.

We have now gathered observations which lead us to dismiss the idea that monotony *per se* leads to anorexia or other forms of nutritive failure in our animals, despite the comment which this feature has received from other investigators. There is no convincing reason why a continued unvaried diet should necessarily be unphysiological; one need only recall the fact that the diet of all sucklings is the same from day to day, and that many of the domestic animals are satisfactorily maintained on rations which are scarcely altered in qualitative make-up except at long intervals. We have observed rats in the same cage for considerably more than a year, during which the daily diet was invariably furnished in the form of our food-pastes. In some of these the composition of the paste was practically the *same* during these very long periods (cf. Charts XXVII, XXVIII, XXIX, XXX). It is true that we could point to many failures to maintain rats on an unchanged diet continued over much shorter periods. One must not, however, here confuse monotony with the real cause of decline. In these latter cases some deficiency or defect in the monotonous feeding sooner or later brings on a physiological state where anorexia occurs; and the advantage which a change of diet initiates ought primarily to be ascribed to the alteration in the food ingredients rather than the relief from the sameness of the intake.

Among factors referring more directly to the nature of the food itself, the *physical texture* and *digestibility* of the nutrients must be taken into consideration. The structure of the food materials may, under ordinary conditions of diet, influence its utilization in no small degree; and the low "coefficients of digestibility" shown by many foods of plant origin testify to this fact. In our experiments the products fed were isolated and reduced to a state of very fine comminution. At most, therefore, some inherent indigestibility of the individual foodstuffs employed might be concerned. Experiments

by M. S. Fine,* while they do not completely do away with this possibility, make it more evident than before that incomplete digestion is, in the case of plant products, for the most part associated with the peculiar vegetable tissues therein contained, rather than a specific resistance of the isolated nutrients.

The need of "*roughage*" to facilitate the normal evacuation of the gut has also been debated. We have, as a general procedure, added the indigestible polysaccharide carbohydrate agar-agar to food-pastes in order to approximate more nearly the conditions which prevail where cellulose enters into the mixed dietary. It can not be maintained, however, that this is necessary for satisfactory nutrition; for we have maintained animals over a year on foods (cf. Chart XXIX) devoid of indigestible principles, if perhaps an exception be made of some of the inorganic ingredients. It is well known that inorganic salts, notably bone ash, may exert the same influence as cellulose in giving bulk to the fæces; and they are often so employed in the technique of metabolism experiments at the present time.†

Aside from the proteins, in which our experimental interest has been primarily centered, our attention has been drawn more and more to those components of the diet which are not sources of energy, yet fundamentally indispensable—namely, the *inorganic compounds*. It is possible that further investigation will compel the inclusion of some of the more vaguely defined and unknown members of the groups spoken of as extractives, lipoids, etc., in this category. Every attempt made by us to approach the solution of the problem of inorganic salts in the dietary has brought fresh surprises.

When Forster‡ fed dogs and pigeons on salt-free foods he made the interesting observation that the animals speedily died—more rapidly even than when all food was withheld. He concluded:

> Der im Uebrigen in Stickstoffgleichgewicht sich befindende thierische Organismus bedarf zu seiner Erhaltung der Zufuhr gewissen Salze; sinkt die Zufuhr unter einer gewisse Grenze oder wird sie gänzlich aufgehoben, so gibt der Körper Salze ab und geht daran zu Grunde.

The classic experiments of Lunin§ on mice led to a somewhat different interpretation of the need of salts. He showed that the animals survived longer on a diet containing an addition of sodium carbonate to the ash-free food than when sodium chloride was added. In the latter case the duration of life corresponded approximately with that observed on a salt-free dietary. From these facts it was argued that the foremost value of the sodium lies in its capacity to neutralize the acids (sulphuric, phosphoric) formed in the metabolism

*M. S. Fine: Dissertation, Yale University, 1911 (unpublished). Cf. Mendel and Fine: Journal of Biological Chemistry, 1911, vols. x and xi.

†Cf. Lothrop: American Journal of Physiology, 1909, xxiv, p. 297.

‡Forster: Zeitschrift für Biologie, 1873, ix, pp. 297–380.

§Lunin: Zeitschrift für physiologische Chemie, 1881, v, p. 31.

of proteins. Sodium chloride obviously has no potential neutralizing power. If the usefulness of the salts were associated solely with their specific character as salts, the salts of sodium ought to be somewhat comparably efficient.

The *function of the inorganic salts* is by no means exhausted, however, by the simple action of chemical equilibrium. It would lead us too far afield in this place to discuss the problem in detail. Charts XI, XII, and XIII, Part I, pp. 38–39) showing the marked differences induced by alterations in the inorganic salts of the diet, the other food components remaining unchanged, are highly suggestive. We have since then made numerous attempts to improve upon the salt mixture empirically selected and prepared somewhat in imitation of the ash of milk. Rats were kept alive (while they steadily declined) 84 days on a food mixture which analysis showed to contain only minimal, inevitable traces of ash (0.16 per cent, a considerable part of which was phosphoric acid derived from the casein). Chlorides were entirely lacking, distilled water being furnished for drinking. In view of this it is necessary to proceed with extreme caution in drawing conclusions from observations extending over brief periods. We shall refer to the subject again, it being sufficient here to emphasize the subtle and specific value of the salts. The lack of knowledge in this field has furnished an obstacle which we have only lately succeeded in overcoming in part.

Even when all these varied conditions are taken into account, there still remain, as we have pointed out before, *extraneous incidents* and *accidental factors* apart from nutrition itself, which may complicate or vitiate experiments like those projected. Disease, old age, injury, may be mentioned in illustration. Failures to maintain nutrition successfully under such extreme conditions do not necessarily imply a deficiency or inadequacy of the dietary. Accordingly, successful experiments must be given far greater weight than failures, where so many possibilities of detrimental influences, aside from the diet itself, are liable to arise over prolonged periods of observation. Some of the uncertainties have been eliminated by the experience previously gained. For example, the intercurrent diseases of our animals have been almost entirely excluded by the use of rats raised in the laboratory for this research. By the prompt elimination of diseased animals, by scrupulous attention to the conditions of the cages and feeding arrangements—in other words, by painstaking attention to hygienic factors—we have succeeded in maintaining a large number of animals in exceptionally good health, so that they have become the more suitable to permit of accurate conclusions regarding the effects of the diets studied. Furthermore, the age and hereditary factors in our animals are now known to us, so that another source of uncertainty has disappeared.

EARLIER EXPERIENCES OF THE AUTHORS.

As the result of the first year's experiments, it was found possible to maintain rats in health and apparent nutritive equilibrium over considerable periods of time on a mixture of isolated food-substances containing isolated proteins as the source of nitrogenous intake. For example, one protocol (Chart XXX) shows that a full-grown rat* was maintained satisfactorily in this way for more than 217 days on glutenin, the animal continuing on this régime at the time when the earlier report was prepared for publication. Rats were likewise maintained on diets in which other proteins, notably casein alone or in combination with isolated vegetable proteins, formed the sole nitrogenous food component, over periods of time exceeding any previously reported, at least under conditions in which the "purity" of the dietary substances was carefully maintained unchanged over equally long periods of time. By maintenance we do not merely mean that the animals remain alive. No feeding experiment is to be regarded as successful in fulfilling the nutritive requirements unless the animals approximately maintain their weight and health (or make normal growth if they are at a stage where this is still to be expected).

Although these apparently successful experiments indicated that the combinations of isolated food-stuffs employed satisfied the nutritive requirements of the rats and consequently constituted a complete food for the maintenance of mature animals, a prolongation of the observations has led to a less favorable outcome. A continuation of the experiments over longer periods has shown that in every case, sooner or later, the animal declined; and unless a change in the diet was now instituted within a comparatively short time the animals died. The Charts XIV, XV, XVI in our earlier paper illustrate this very well. The rats 23, 24, 25 were maintained without noteworthy alterations in weight over 130 to 160 days on a constant mixture including a single protein. The animals ate well, as the food records show, until the final period of decline.

These records can be duplicated, especially in respect to the decline, by many others, as for example Charts XLI, XLII, LXXVIII, LXXIX, LXXX, CII, CXV, CXVI appended to this report. The history of rat 71 is particularly instructive on this point.† This animal (see Chart XXX), weighing 257 grams on April 5, 1910, was put upon a diet containing casein (12 per cent) and glutenin (6 per cent) as the only proteins. Subsequently glutenin alone (16.4 per cent after 69 days and 18 per cent after 104 days) formed the protein of the diet. The rat continued in excellent nutritive

*The earlier data regarding this animal, rat 71, are given in Publication No. 156, Carnegie Institution of Washington, p. 47 ff.

†The earlier data will be found in Publication No. 156, Carnegie Institution of Washington, pp. 47–48.

condition, eating well and exhibiting favorable nitrogen balances, until the end of $9\frac{1}{2}$ months, when a gradual decline was observed. When the animal, at the end of a total feeding period of 335 days (42 days after the onset of the decline) was reduced to 162.5 grams in weight and near death, an attempt was made to see whether the decline was due solely to improper food or to the onset of old age or disease. With mixed food realimentation took place at once and the rat regained its weight in a week. A resumption of the former glutenin food during 35 days gradually led to a second decline, which was promptly checked by a change in the diet involving only the non-protein components of the food mixture. Here, then, is a record of the feeding of a full-grown rat, with the exception of 7 days, during a period of 454 days on a diet of isolated food-stuffs and on a diet containing a single protein, glutenin, for 371 days. This observation is remarkable because of the exceptional duration of the experiment. It is apparent, therefore, that *as a maintenance diet our food lacked something other than protein and energy.*

It remains to be shown precisely what the lacking component of our earlier diets is, whether some organic constituent or a peculiar proportion of inorganic ingredients. In any event it is evident that our original artificial food mixtures are incapable of supporting life indefinitely. Aside from this, however, records like that of rat 71 living on glutenin as the sole source of protein (see Chart XXX), or rat 133 (Chart LXX) on edestin, in contrast with rats XI, XIV, 146, and 157 (Charts CXXVI, CXXVII, CXXVIII, and CXXIX) on zein indicate the possibility of nutritive inequalities among the proteins themselves. Marked deficiencies tend to manifest themselves in comparatively short periods of time. In all of these cases the food actually consumed supplied sufficient *energy* for the immediate needs of the rats under investigation.

In the continuation of our experiments we have tried to profit by the first year's experiences. The methods have not been materially altered, except that the determination of the *nitrogen balance* has been omitted for the present. We learned from very numerous trials that it runs parallel with gain or loss of weight, and that the food intake varies closely with the weight of the animal, thereby making a record of the nitrogen unnecessary for judging the nutritive status of the rats employed. The same cages as heretofore have continued to prove very satisfactory. Instead of being rested on glass funnels for the collection of urine, they are now placed over a frequently changed sheet of absorbent paper (paper napkin) upon an enameled tray or pan. The fluid excreta thus promptly absorbed are frequently removed. It has already been pointed out that the food mixtures, prepared in paste form to prevent scattering by the animals and make it possible to obtain accurate records of the quantities eaten,

are not ideal in composition. The inclusion of 20 to 45 per cent of fat in the diet—a condition necessitated by the requirements of the experiments as outlined—seems like an excessive amount; nevertheless the utilization appears to be satisfactory and attempts to devise less objectionable modes of feeding have been unsuccessful in our hands.

ALIMENTARY BACTERIA AND NUTRITION.

In the course of our later studies we have been forced to take cognizance of the possible rôle of the bacterial flora of the alimentary tract in relation to appropriate nutrition. The water-free, fat-rich food characteristic of our experimental dietaries is not, broadly speaking, a particularly favorable medium for the development of certain groups of bacteria. The food of our animals therefore probably introduces into the digestive tube of the experimental animals bacterial invaders somewhat different from those which normally inhabit the alimentary tract of rats living on a free mixed diet. It is quite conceivable, therefore, that the bacterial conditions may be altered markedly as a result of the restriction in the growth of certain groups or the facilitation of the development of still others in the alimentary tract under these changed and sustained conditions of altered diet.* It is well known, for example, that in higher animals the preponderance of acid-producing organisms—to use a single illustration—may lead to an inhibition of the growth of the putrefactive group.

Guided by such considerations and the observation that those rats that have been maintained for long periods on diets with isolated food-stuffs become koprophagists, we have initiated the plan of feeding small quantities of the fæces of rats living on ordinary mixed food to some of our experimental animals, particularly in cases where symptoms of nutritive decline had become manifest. In nearly every instance the occasional addition of a small amount of the fæces from a normally fed rat at once stopped the decline in weight of the experimental animals to which a single protein was being fed. The results in almost all of these cases have been sufficiently striking to warrant a further pursuit of this topic. In our experiments there appears to be an unmistakable favorable influence induced by the occasional addition to the dietary of normal fæces with their high bacterial content. It must not be overlooked that other components besides bacteria, notably inorganic salts and unknown compounds, are also furnished by this means; but the quantities involved have always been very small. Further investigation will be necessary and is already projected.

The procedure in the case of these fæces-feeding trials consisted in introducing small amounts (about 0.5 gm.) of air-dry excrement

*Cf. Herter and Kendall: Journal of Biological Chemistry, 1910, VII, p. 203; Kendall: Journal of the American Medical Association, April 15, 1911.

of rats on mixed food into the cages twice a week. It is an interesting observation that when the rats kept on a mixture of isolated food-substances were offered a choice between their own fæces and those of rats on mixed diets, they invariably chose the fæces of the latter. In many cases we have noticed a marked improvement in the nutritive conditions of animals maintained on a single-protein dietary when other rats were introduced into their cages for breeding purposes. In view of the favorable influence exerted by feeding the fæces of rats living on mixed food, it is quite likely that the presence of the strangers in the cages furnished a suitable opportunity to obtain "normal" fæces. This may explain the favorable results noted, in contrast with the negative effects seen where several rats living on the same single-protein diet have been maintained in the same cage.

The extent of the influence exerted by what we have, in the absence of a better explanation, assumed to be bacterial influences, is illustrated in some of the appended charts, the periods at which the fæces feeding was begun being indicated. The favorable effects have not been confined to experiments with one protein, but are manifested with casein (see Charts XXXIX, XL, XLI, and XLII), with edestin (see Charts LXVI, LXVII, LXVIII, and LXIX), and with gliadin (see Charts CI, CII, and CIII). Two failures may likewise be recorded, viz, an ultimate one with casein (Chart XLI) and a complete one with edestin (Chart LXXVII) as the protein component. These were not due to incapacity of the animals to grow, since further alteration of diet brought marked improvement.

The influence of fæces feeding is especially striking in the case of the gliadin tests, since without the addition of the fæces it has been almost impossible to attain satisfactory nutritive condition with this protein plus the special non-protein components of the food here employed. It is instructive therefore to compare such failures (cf. Period 2, Charts CXV and CXVI) with Charts CI and CIII, in which fæces feeding was resorted to.

In four of the experiments with edestin-food alluded to and recorded on Charts LXVI, LXVII, LXVIII, and LXIX, fresh fæces were not actually introduced into the cages; but the improvement, and even growth, in these young rats is coincident with the opportunity afforded to obtain "normal" fæces when other rats were daily introduced into the cages for a few hours.

In Chart CII is seen the result of an attempt to determine whether the favorable influence of the fæces is actually of bacterial nature. Fæces were fed as in the comparable gliadin experiments (Charts CI and CIII); but they were previously sterilized by thrice repeated heating in an atmosphere of steam. The decline of the animal was not prevented to the same extent with sterilized as with normal fæces. Further trials are necessary in this direction; and our

experience, though limited, invites attention anew to the possible nutritive functions of bacteria in the alimentary tract. Some of the aspects of this problem are referred to in our earlier paper.*

NUTRITION AND GROWTH.

The criteria of adequate nutrition are quite different in the case of *growing* animals from those applying to adults of the same species. During the period of adolescence it is not sufficient to maintain a condition of nutritive equilibrium and constancy of form or body-weight. In this stage of an animal's existence there should be evidences of development, and *growth* should manifest itself in a change of size. The curve of growth, expressed in changes of body-weight, is remarkably constant and characteristic for each species under the ordinary conditions of nutrition and environment. The individual values may at times fluctuate about a mean; but in the majority of cases the excursions from the average are not extensive.

In Chart XXII are reproduced curves illustrating the average normal rate of growth of the white rat, both male and female. The statistics for two of the curves are taken from Donaldson,† whose observations we have repeatedly verified in their general features. A third curve on the same chart represents the results of our own observations on the growth of the female white rat, regarding which data are less abundant. It will be noted that the curves of growth for the two sexes do not completely coincide in type. After an age of 70 days, represented by a body-weight of about 100 grams, the rate of growth is somewhat slower in the female than in the male. Indeed, the females rarely attain the large weight and size exhibited by the normal adult males of the same age, even in the case of animals from the same litter. We gain the impression that our "breed" of rats may in general be somewhat smaller than those measured by Donaldson and his collaborators. At any rate, the data available for statistical purposes are not very extensive and the curves here presented must have only a provisional value until more numerous measurements are made. In connection with certain of our experiments it may be stated that "the effect of mating on the growth-curve for the males can probably be neglected."‡ In the case of females, the effect of the bearing of young is, according to Watson,§ "to render the mated rats slightly heavier than the unmated—some of the excessive weight being due to the larger amount of fat present in the mated animals." Two charts (XXIV, XXV) are appended

*Carnegie Institution of Washington, Publication No. 156, p. 3.
†Donaldson: A comparison of the white rat with man in respect to the growth of the entire body. Boas Memorial Volume, New York, 1906.
‡Cf. Donaldson: *ibid*, p. 8.
§Watson: Journal of Comparative Neurology, 1905, xv, p. 523.

to illustrate the influence of the course of pregnancy on the growth-curve of female rats of different sizes.

Making allowance for these minor divergencies, the striking uniformity in the progress of development in an animal nevertheless is a specific racial characteristic, and gives to the *curve of growth* a unique value as an index of the conditions which determine it. Growth is affected by two factors: nutrition, and what Rubner has termed "Wachstumstrieb" or growth-impulse. The latter factor is inherent in the animal. The limits are determined by heredity and can not be altered materially by the most abundant diet. "Eine noch so reichliche Ernährung vermag die in der Rasse und deren Vererbung gelegenen Grössen- und Massenbegrenzungen nicht zu mehren."*

We are not prepared, at this time, to discuss the nature of the hereditary factor or impelling "force" in growth.† Aron writes:

> Die Natur des Wachstumstriebes ist dunkel. Sie ist eine Funktion der Zellen, im besonderen der jugendlichen Zellen. Welche Faktoren diesen Zelltrieb regulieren, wissen wir nicht, vor allem nicht, warum er allmählich aufhört. Ob hier die Zeitdauer seiner Wirksamkeit, ob die erreichte Grösse des Individuums den Ausschlag für das Abklingen des Wachstumstriebes gibt, ist bis jetzt nicht entschieden.‡

Rubner has attempted to formulate its character:

> Die eine grosse Unbekannte auf dem Gebiete der Wachstumsphysiologie ist der *Wachstumstrieb*, der in gesetzmässiger Weise den Gang der Entwicklung, Massenzunahme, durch die Regelung der Ernährung leitet. Den *Urgrund* hat dieser Wachstumstrieb in der Geschwindigkeit der Kernteilung; wie wir noch sehen werden leitet sich hieraus der ganze Prozess des Stoffumsatzes ab. Die Kernteilungsgeschwindigkeit ist offenbar etwas der Spezies Eigentümliches, somit sind wir nicht in der Lage, vorläufig tiefer in dieses Problem vorzudringen.§

The second factor in growth, namely, *nutrition*, can be approached more easily by the experimental method. It is along this line that we have hoped, therefore, to be able to attack some of the problems of the relative value of the individual foodstuffs. It is well known that growth can be retarded by means involving the nutrition of the individual. Waters has well summarized the situation in these words:

> The upper limit of the size of an animal is determined by heredity. The stature to which an animal may actually attain, within this definitely fixed limit, is directly related to the way in which it is nourished during its growing period. Some of our approved theories have been so extreme as to hold, in effect, that the animal must grow at its maximum rate practically every

*Rubner: Archiv für Hygiene, 1908, LXVI, p. 82.

†Certain aspects are considered in C. S. Minot: The problem of age, growth, and death. New York, 1908.

‡Aron: Biochemische Zeitschrift, 1910, XXXp. 207.

§Rubner: Archiv für Hygiene, 1908, LXVI, p., 86.

day from birth to complete maturity in order to reach its normal size, or the full stature fixed by heredity. In other words, it is assumed that the animal has but one way of reaching its full stature and full development, viz., by developing to its upper limit through its entire growth period. This assumes that the organism is utterly incapable of compensating for any retarded development at any time in its growth period, either by a subsequently increased rate of growth, or by extending, even in the slightest degree, the growth cycle, much less by growing for a time at least when so sparsely fed that no gain in weight occurs.*

Rubner has expressed the rôle of nutrition in growth as follows:

Kann die Ernährung auch keinen Wachstumstrieb schaffen, so kann sie, wenn ungünstig und unzweckmässig, doch zu einem *Hemmnis* des natürlichen Wachstums werden. Wachstumsbehinderung ist innerhalb gewisser Grenzen noch keine Ursache einer Existenzgefährdung, ein Kind, dem die Nahrung normales Wachstum hindert, stirbt deswegen durchaus nicht, es holt später leicht wieder ein, was es versäumt hat . . . Nur das steht sicher, dass die Behinderung des Wachstumstriebes, wie dies wirklich vorkommt, nicht während der ganzen Wachstumsperiode andauern darf, da sonst allerdings die Grösse des Individuums dauernd Schaden leidet. Verlorene Körpergrösse in der Jugendzeit kann nach Vollendung der Wachstumsperiode nimmermehr abgeglichen werden . . . Eine *optimale* Ernährung, wie die *Wachstumsernährung* sein muss, stellt an die richtige Auswahl der Stoffe ganz andere Anforderungen als eine einfache Erhaltungsdiät.†

Obviously the energy problem plays an important part in the nutrition of growing animals. For the present we are primarily concerned with the qualitative aspects of the diet rather than the quantitative features of the food-intake. These two factors may at times stand in intimate relation to each other; improperly constituted food may, for example, modify the amount eaten and therefore the energy available for growth. As was intimated in our first report we have been able to arrest development in rats by feeding mixtures containing a single protein; but inasmuch as the food intake was not measured at that time, it was impossible to say whether the chemical character of the diet or a quantitatively inadequate food consumption was responsible for the dwarfing. The *fact* brought out was that in these young animals there could be a *maintenance of weight*, precisely as in older rats.

Waters has appropriately emphasized the necessity of a more exact definition of what is meant by *maintenance*, in contrast with growth. He writes:

It has long been assumed that the body of an animal, when supplied with only sufficient nutriment to maintain its weight, remains constant in composition and that no growth or production or change of any sort occurs.

*H. J. Waters: The capacity of animals to grow under adverse conditions. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 3.

†Rubner: Archiv für Hygiene, 1908, LXVI, pp. 82–83.

It is true that the term *maintenance* has been used somewhat loosely, but in general we have been in the habit of regarding the animal in maintenance when its live weight was constant. A more correct definition of the term would perhaps be to say that the animal was in maintenance when its body was in energy balance, but the live weight has been the conventional measure of our maintenance values.*

It is generally admitted that the proteins satisfy several functions in a growing organism as well as in the adult. The first is that of maintenance, corresponding with what has been termed the "Abnutzungsquote," or wear-and-tear, by Rubner. This makes good the inevitable losses occasioned by the processes of metabolism, cellular and secretory processes, etc. It is a small yet ever present need for protein (as well as energy), representing in a general way the minimal protein need of the stationary organism. Any excess of protein beyond this maintenance requirement may, in the adult, experience temporary storage ("Ansatz") or be devoted to dynamogenic purposes; but in the organism capable of development it contributes a share toward growth. It should be emphasized that the rate of growth is not by any means proportional to the excess of protein available. It is surprising, indeed, how small a content of protein in the dietary suffices to make growth possible. Rubner and Heubner† found, for example, that in suckling infants a protein intake equivalent to 5 per cent of the total calories satisfies the protein needs of maintenance, while 7 per cent permits of growth. Rubner writes:

> Das Wachstum ist eine *Funktion der Zelle*, es kann durch unzureichender Eiweisszufuhr *latent* werden, aber Eiweiss vermag *nicht* die Wachstumsschnelligkeit über die von der Natur gestreckten Grenzen zu heben, daher wird mit steigender Eiweissmenge in der Kost prozentisch weniger verwertet und das überflüssig zugeführte Eiweiss wird einfach als Brennstoff verbraucht der isodyname Mengen N-freier Stoffe einspart. Diese starke Anziehung von Eiweiss zum Wachstum nimmt im Laufe der Entwicklung ab und ist am grössten in der ersten Zeit des Lebens.‡

Waters has found in his extensive studies on cattle that growth, in the sense of changes of size and form, may occur even under adverse nutritive conditions. Fundamentally such investigations touch upon the much controverted question as to the relative importance of breeding and feeding in determining the shape and activities of mature animals. It is well known that by limiting the food supply of an ungrown individual, its development may be retarded. If the underfeeding is prolonged through the cycle of growth, the full stature limited by heredity may not be reached.

*H. J. Waters: The capacity of animals to grow under adverse condition. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 3.

†Rubner and Heubner: Zeitschrift für experimentelle Pathologie, 1905, I, p. 1.

‡Rubner: Archiv für Hygiene, 1908, LXVI, p. 110.

Waters asked the question:

Will this animal of smaller stature be in the same proportion with respect to all the organs and the different parts of its body as though it had been nourished to its full capacity and had attained its normal size and maximum development? Or will in this period of sparse nourishment a more complete development occur in certain parts of the body than in other parts? In short, when there is not sufficient food supplied to the growing animal to develop all of the organs and all parts of the body to their full limit and extent, will the rate of development of certain of these organs or parts diminish earlier than others and will the development of certain parts cease altogether before the development of other parts is diminished in rate and is it possible that some parts may cease their development before that of other parts?*

In actual experiments at the Missouri Agricultural Experiment Station, Waters found that ungrown cattle may remain at a constant body-weight for a long period of time, and yet increase in height and apparently decrease their store of fat. In other words, the skeleton has grown, or at least the bones have lengthened. Two interesting illustrative protocols† are reprinted here, one, Table XXXI, in which a stationary body-weight was maintained, the other, Table XXXII, in which there was actual decline on a starvation ration.

TABLE XXXI (FROM WATERS, TABLE II).—SHOWING INCREASE IN HEIGHT AT WITHERS, LENGTH OF HEAD, DEPTH OF CHEST, WIDTH OF CHEST, AND LOSS OF FAT IN A YEARLING STEER WHEN KEPT AT A STATIONARY BODY-WEIGHT.

No. 595. Grade Hereford. Born May 15, 1907. Nine and a half months old when experiment began. Full fed four months previous to beginning of trial. Condition when put on maintenance, medium. Weight at beginning of trial, 609.2 lbs. Weight at close of trial, 595.6 lbs. Average of ten daily weights.

Date.	Height at withers.	Length of head.	Depth of chest.	Width of chest.	Condition.
1908.	*cm.*	*cm.*	*cm.*	*cm.*	
Feb. 8	109	38	56	35	Medium.
Mar. 13	112.5	40	58	36.5	Medium.
Apr. 11	115.5	41	57.5	35.5	Medium to thin.
June 2	116	42	59	33.5	Common.
July 1	117.5	44	58.5	34	Common.
Aug. 1	117.5	44	59	33	Common.
Sept. 2	117.5	44	59.5	33	Common to fair.
Sept. 29	119	45.50	59.5	33.5	Fair.
Oct. 30	119.25	45.75	59.5	31	Fair.
Nov. 30	119.5	45.75	59.5	31	Fair to thin.
1909.					
Jan 1	119.75	46.50	60.5	30.75	Thin.
Jan. 30	119.75	45.50	60.75	30.75	Thin.
Total height in 12 months.	10.75	7.50	4.75	*— 4.25	
Per cent gain	9.86	19.73	8.48	*—12.1	

NOTE.—When slaughtered, carcass was classed as poor canner. All visible subdermal and intramuscular fat had disappeared.

*—Denotes a loss.

*H. J. Waters: The influence of nutrition upon the animal form. Proceedings Society for the Promotion of Agricultural Science, 1909, XXX, p. 71.

†From H. J. Waters: The capacity of animals to grow under adverse condition. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX.

TABLE XXXII (FROM WATERS, TABLE VI).—SUB-MAINTENANCE.

Steer No. 591. Grade Hereford. Born May 15, 1907. Experiment began Feb. 26, 1908.
Age of animal at beginning of experiment, nine and a half months.
Full fed four months before trial began and was in good condition.
Weight at beginning of trial, 572.7 lbs. Weight at close of trial, 490.4 lbs.
Total loss in weight, 82.3 lbs. Average daily loss 0.43 lb. —Denotes loss.

Date.	Height at withers.	Length of head.	Depth of chest.	Width of chest.
1908.	*cm.*	*cm.*	*cm.*	*cm.*
Feb. 8	110.5	39	57	38.5
Mar. 13	113	41.5	57.5	34.5
Mar. 28	115	42	....	35
Apr. 11	114.5	41	58	33
May 2	116	42	57	33
June 1	118.5	44	57.5	33
June 29	120	44	58	31.5
July 31	119	44.5	59.5	29.5
Aug. 31	119.5	44.5	58	29
Gain	9	5.5	1	− 9.5
Per cent	8.14	14.10	1.75	−24.6

The following is from Waters, in regard to a series of comparable cattle maintained by him on different nutritive planes, designated as sub-maintenance, maintenance, and super-maintenance:

It is to be observed that there is no appreciable difference in the rate of growth in height of these three animals on widely different nutritive planes, from the beginning of the experiment (February) to the end of June. At this time the curve of the sub-maintenance animal flattens perceptibly. A month later, the maintenance animal is apparently responding to the influence of the low nutritive plane. As would be expected, in the case of the super-maintenance animal, the rate of growth remains unchanged. It may be surprising to many [Waters writes elsewhere] that an animal on maintenance, much less on sub-maintenance, should show any increase whatever in the width of hip or length of leg . . . Apparently the animal organism is capable of drawing upon its reserve for the purposes of sustaining the growth process for a considerable time and to a considerable extent. Our experiments indicate that after the reserve is drawn upon to a considerable extent to support growth the process ceases, and there is no further increase in height or in length of bone. From this point on the animal's chief business is to be to sustain life. This law applies to animals on a stationary live weight as well as those being fed so that the live weight is steadily declining, and indeed to those whose ration, while above maintenance and causing a gain in live weight, is less than the normal growth rate of the individual. Such an animal will, while gaining in weight, become thinner, because it is drawing upon its reserve to supplement the ration in its effort to grow at a normal rate.*

More recently Aron† has made comparable studies on growing dogs. He formulated his problem in the following words:

"Was wird geschehen, wenn für kürzere oder längere Zeit in der Nahrung nur so viel Energie usw. zugeführt wird, wie erforderlich ist, um den Erhalt-

*H. J. Waters: How an animal grows. Kansas State Board of Agriculture, Seventeenth Biennial Report, 1909–1910, I, p. 208.

†Aron: Biochemische Zeitschrift, 1910, XXX, p. 207.

ungsbedarf des wachsenden Organismus zu befriedigen, aber kein Ueberschuss, der als Wachstumsenergie dienen könnte? Die nächstliegende Annahme ist, dass dann kein Wachstum stattfindet, dass der Wachstumsprozess stillsteht. Können wir nun wirklich den Wachstumstrieb durch Nahrungsbeschränkung unterdrücken? Wie lange? Und was geschieht später mit einem wachsenden Organismus, dessen Wachstum eine Zeitlang hintan gehalten worden ist? (p. 208.)

Aron succeeded by restricted feeding in attaining constancy of body-weight in practically all of his dogs, in some cases during a period of nearly a year. The daily gains or losses fluctuated within a few grams. The description of the animals during the experiments is of interest to us:

Bei allen Hunden konnte man deutlich beobachten, wie die Tiere trotz des Gewichtsstillstands *wuchsen*, d.h. an Höhe und Länge zunahmen. Dabei wurden die Tiere zusehends magerer, Fett und Muskeln schienen an Masse abzunehmen, die runden Formen schwanden, die Knochen traten eckig unter der Haut hervor, und schliesslich schienen die Tiere nur noch aus Haut und Knochen zu bestehen. Trotzdem waren die Hunde nicht etwa schwach. Im Gegenteil, sie waren lebhaft, liefen und sprangen umher, oft mehr als ihre normalen Brudertiere, die ein zwei- oder dreimal zo grosses Körpergewicht zu bewältigen hatten. Dieser Zustand zunehmender Abmagerung unter ständiger Grössen-, d.h. Längen- und Höhenzunahme bei Konstantbleiben des Gewichtes dauerte je nach dem Grade der Nahrungsentziehung ungefähr 3 bis 5 Monate an. Wurde jetzt, wenn das Tier völlig abgemagert war, . . ., die Nahrungsmenge *weiter so gering* belassen wie vorher, so ging das Tier unter geringem Gewichtsverlust in völliger Inanition zugrunde. Wurde aber jetzt die Nahrungsmenge etwas erhöht, wie bei Hund A, so hielt sich das Tier zwar vollkommen abgemagert, aber auf konstantem Gewicht. *Und jetzt erweist sich dieser Gewichtsstillstand als identisch mit Wachstumsstillstand!* Der Hund A ist noch weitere 5 Monate auf dem gleichen Gewicht gehalten worden, ohne dass sich nun in seinem Aussehen nennenswerte Aenderungen konstatieren liessen.

Durch geeignete Nahrungsbeschränkung gelingt es also, wachsende Hunde beliebig lange auf konstantem Gewicht zu halten. Natürlich darf man nicht allzu junge Tiere nehmen. Während dieses Gewichtsstillstandes gehen aber gewaltige Umwandlungen im Tierkörper vor, die sich äusserlich in dem fortschreitenden Längen- und Höhenwachstum und der Abmagerung dokumentieren.

Offenbar ist trotz des Gewichtsstillstandes das Skelett weiter gewachsen und hat nicht nur an Grösse, sondern auch an Masse zugenommen. Folglich müssen andere Körpergebilde (wie Haut, Fleisch, Organe usw.) an Gewicht verloren haben; denn sonst könnte ja das Gewicht des Tieres nicht das gleiche geblieben sein. Ebenso wie die Massenverhältnisse der einzelnen Körpergebilde haben sich nun höchstwahrscheinlich auch die Mengenverhältnisse der einzelnen Körperbestandteile, wie Fett, Eiweiss usw., beträchtlich verschoben. (p. 212.)

Aron's analyses of the underfed dogs showing stationary weight in comparison with well-fed control animals indicate that in addition to the bones, the brain also was protected from loss of weight, while the adipose and muscular tissue suffered notable losses. Most striking is the degree to which water has replaced the tissue substance

utilized to compensate for the lack in the food, the blood especially becoming distinctly "watery," as the selected protocol shows:*

TABLE XXXIII.—CONTENT OF DRY MATTER IN VARIOUS TISSUES.

	Control dog.	Underfed dog.
Blood	*18.8	*5.1
Brain	24.6	19.3
Bones	57.2	40.0
Muscle	29.1	15.2

*Protein = N×6¼.

It is apparent here, as in Waters's experiments, that the energy deficit has been furnished by the body. "Sind alle verfügbaren Reservestoffe aufgebraucht, dann gewinnt der Erhaltungstrieb die Oberhand über den Wachstumstrieb, und das 'Wachstum' stockt." (Aron, p. 222.)

In relation to our own later observations it is desirable to quote Aron's view regarding the impulse to growth. He concludes:

> . . . dass die innere treibende Kraft zum Wachsen überhaupt in dem Kerngerüst des Körpers, dem Skelett, ruht. Die Muskulatur verfügt anscheinend über gar keinen richtigen Wachstumstrieb. Sie folgt dem wachsenden Skelett nur dann, wenn die Ernährungsverhältnisse es erlauben, vielleicht auf Grund rein mechanischer Kräfte (Zug).
>
> Recht interessant scheint zum Schluss noch die Frage, wie sich bei den durch lange fortgesetzte Unterernährung im Wachstum zurückgehaltenen Tieren die Entwicklung und die Entwicklungsfähigkeit verhält. Mein Tiermaterial war nicht ausreichend, um ein Studium der Geschlechtsorgane der zwar im Alter der Geschlechtsreife stehenden, aber im Wachstum weit zurückgebliebenen Tiere zu gestatten. Dagegen scheint mir die Beobachtung der *Stimme* auf ein wirkliches Zurückbleiben der Entwicklung auf dem infantilen Stadium zu deuten. Die Unterschiede zwischen den Brudertieren der ersten, zweiten und vierten Versuchsreihe waren auffällig. Die im Gewicht zurückgebliebenen Tiere schrien kreischend wie junge Hunde, während ihre normalen Brudertiere mit tiefem Tonfall bellten. In ganz dem gleichen Sinne spricht die von Waters festgestellte Tatsache, dass seine in Gewicht und Wachstum zurückgebliebenen Tiere ein Fleisch, das für 'Kalbfleisch' charakteristisch war, aufwiesen, während sie dem Alter nach schon "Rindfleisch" besitzen sollten. (pp. 222–223.)

Studies of the relation of weight to the measurements of children during the first year† have also given evidence of "disproportionate" growth in the case of poorly nourished infants. Whereas there is, in the normal infant, a fairly constant relationship between body-weight and height, circumference of head, chest, etc., this is not true where proper increase of body-weight is retarded by poor nutrition. For example, in children whose weight at the end of the third month

*Aron: Biochemische Zeitschrift, 1910, xxx, p. 220.
†E. C. Fleischner: Archives of Pediatrics, October 1906.

is only equal to that of a normal child at birth, the height has been found above that of the latter, illustrating, as Fleischner remarks, "that age plays some part in the growth of the infant, independent of the weight." This corresponds with the cases of the animals already cited. Fleischner concludes from his measurements of 500 children of whom 25 per cent were well nourished, 35 per cent fairly well nourished, and 40 per cent poorly nourished:

> It is in the poorly nourished children that age plays its most important part . . . In the poorly nourished children, most of whom are probably somewhat premature, when the weight is below normal, all the measurements are correspondingly below normal. The height and circumference of the head reach the normal birth measurements a little ahead of the weight, while the chest and abdomen are two months later in reaching the measurements of a normal child at birth. When the weight is stationary the increase in the measurements is very small, depending upon the slight influence which age has upon the growth of the infant notwithstanding the weight. The measurements of infants of the same weight, notwithstanding the age, are very similar, the small difference depending, as when the weight of a child is stationary, upon the very slight influence of age upon growth. The final conclusion can be drawn that during the first year of life the primary factor in the increase of the measurements of the body is steady, consistent increase in the weight, the influence of age being secondary and much less important.*

SUSPENSION OF GROWTH ON A MAINTENANCE DIET.

Early in the course of our investigation we noted that young rats could remain in apparent good health while living on some of the mixtures of isolated food-stuffs, without giving any evidence of growth. In some instances the animals ultimately declined and died where the diet was not changed; but in numerous cases body-weight, which we used as our guide, remained practically unchanged or showed a minimal slow increase (cf. Charts XXXVII, LXIII, and LXIV). The experiment showing the greatest growth under these dietary conditions is recorded in Chart XXXVIII. Other investigators have met with this stationary condition and accepted it as evidence of satisfactory nutritive equilibrium. We soon became convinced, however, that a diet which will not induce real growth at the proper age is unquestionably defective from the standpoint of perfect nutrition. Furthermore, inasmuch as the ungrown rat has a far smaller reserve of available energy and manifests the utilization of a suitable diet both speedily and conspicuously by its measurable changes in size, the animal becomes an exceptionally appropriate subject at this early stage for the study of the nutritive requirement.

The most precise evidence which we can present at this time of the stationary condition of the animals which we have stunted by

*E. C. Fleischner: Archives of Pediatrics, October 1906.

the particular dietaries adopted is derived from measurements on three young rats of the same litter maintained for 124 days without noteworthy growth, on a diet of

	Per cent.
Glutenin	18.0
Starch	14.5 to 34.5
Sugar	15.0 to 20.0
Agar	5.0
Salt mixture I	2.5
Lard	20.0 to 45.0

The curves of growth of these animals as well as three others from the same brood fed on mixed food or the milk-food mixture (and showing a normal growth) are reproduced in Charts LXXXI, LXXXII, LXXXIII, LXXXIV, LXXXV, and LXXXVI.

The animals were killed at the age of 178 days and measurements were made by Dr. S. Hatai, of the Wistar Institute. The tabulated data are given on the following page, together with a report from Dr. Hatai, to whom, as well as to Dr. Donaldson, we are greatly indebted for helpful cooperation.

The statistics of body-length, weight of brain, spinal cord, etc., of the stunted animals at an age of 178 days are comparable with those characteristic for normally growing rats of the *same* body-weight, which is attained at an age of approximately 54 to 63 days. Here, then, are illustrations of maintenance without growth.

Dr. Hatai further reports as follows:

Since it seems to be the least variable character, I have selected the body-length as the basis for computation. When the other characters which we can measure are calculated from the formulas based on body-length, it is seen that the observed weight of the brain and of the spinal cord agrees closely with the calculated in both the control and the stunted rats. Thus both series have a growth of the nervous system normal to their body-length. In the control series, the percentage of water observed in both the brain and the spinal cord agrees with that calculated according to the body-length. In general then the control rats agree with the general population in these characters. Since the stunted rats have an abnormally small body-length for their age, they can not be treated by the formula for determining the percentage of water from body-length. When, however, we take the estimated percentage of water for 178 days (see Donaldson*) we find that this value agrees with that observed in the stunted series. It may be further noted that the ratio between body-length and tail-length is the same in both series. We therefore conclude that *in both series the body-weight is normal to the body-length; the brain and spinal cord weight normal to the body-length; and the percentage of water normal for age.* Concerning other organs we have no data, but we may infer from the foregoing that they also have weights normal to the body-length. You will see from the above that the stunted rats though small have the general relative development of the controls and that in the only case where it is possible to follow the maturing process, that is in the percentage of water in the nervous system, they have matured in accordance with their age (see Donaldson*).

*Donaldson: Journal of Comparative Neurology, April 1911.

A
B
C

A. Rat 238, female. Age 140 days, weight 144 grams, which is normal for a rat of same age as 240.
B. Rat 240, female. Age 140 days, weight 55 grams. Same brood as Rat 238.
C. Rat 305. Age 36 days, weight 55 grams. Showing the appearance of a normal rat of same size as 240.
A and B show the contrast between two rats of the same age, one of which (Rat 240) has been stunted. The lower two pictures afford a comparison between two rats of the same weight, but widely differing in age. The older, stunted rat, B, has not lost the characteristic proportions of the younger animal, C.

D

E

F

D. Rat 168, male. Weight 235 grams, which is normal for a rat of the age of 220 shown below.

E. Rat 220, male. Age 148 days, weight 58 grams.

F. Rat 305. Age 36 days, weight 55 grams. Showing appearance of a normal rat of same weight as 220.

D and E show the contrast between two rats of the same age, one of which (Rat 220) has been stunted. The stunted rat is not essentially altered in its bodily proportions from those of a much younger rat of the same weight.

TABLE XXXIV.—HATAI'S MEASUREMENTS OF STUNTED RATS FROM EXPERIMENTS OF OSBORNE AND MENDEL, 1910–1911.

CONTROL RATS.

	Diet.	Sex.	Age in days.	Weight in grams of—			Hypophysis.	Percentage of water.		Length in mm. of—	
				Body.	Brain.	Cord.		Brain.	Cord.	Body.	Tail.
Rat 96...	Milk	Fem.	178	154.9	1.7346	0.4934	0.0073	78.306	71.139	176	146
Rat 97...	Milk	Fem.	178	164.5	1.6974	0.5007	0.0093	78.473	71.220	183	164
Rat 99...	Mixed	Male	178	175.0	1.8515	0.4810	0.0052	78.623	71.809	181	144
Average.......				164.8	1.7612	0.4937	0.0071	78.467	71.389	180	151
Calculated from body-length.........					1.7645	0.5004	......	78.374	71.192	180	.....
Estimated percentage of water from age				.....	.....	.....	......	78.4	71.2	.....	.....

Body-length to tail-length 1 : 0.83

STUNTED RATS.

	Diet.	Sex.	Age in days.	Body.	Brain.	Cord.	Hypophysis.	Brain.	Cord.	Body.	Tail.
Rat 100..	Glutenin	Fem.	178	85.0	1.6323	0.4089	0.0035	78.141	70.775	148	129
Rat 101..	Glutenin	Male	178	71.8	1.5022	0.3781	0.0022	78.272	71.701	139	108
Rat 102..	Glutenin	Male	178	85.7	1.6280	0.3977	0.0033	78.133	71.134	148	125
Average.......				80.8	1.5875	0.3929	0.0030	78.182	71.203	145	121
Calculated from body-length..........					1.5896	0.3639	.....	.....	.....	145	.....
Estimated percentage of water from age				.....	.....	.....	.....	78.4	71.2	.....	.....

Body-length to tail-length 1 : 0.83

FORMULAS.

$$\text{Brain weight} = 0.569 \log \left(10 \frac{\text{Body-length}+134}{143} - 23.7\right) + 0.554$$

$$\text{Spinal cord weight} = 0.585 \log \left(10 \frac{\text{Body-length}+134}{143} + 6\right) - 0.795$$

Percentage of water (brain) $= 82.62 - 2 \log (\text{Body-weight} - 10)$.
Percentage of water (spinal cord) $= 85.20 - 6.5 \log (\text{Body-weight})$.

Photographs of other rats which have been dwarfed in like ways give evidence of the similarity of the stunts in general appearance with normal animals of the same weight at a much earlier age. Thus, in Plate 1, rat 305, C, weighing 55 grams at the age of 36 days, compares favorably with rat 240, B, dwarfed on a gliadin food mixture, at the age of 140 days, when it weighed 55 grams (cf. Chart CXIII). It is interesting to contrast B with the uppermost photograph A of rat 238, likewise 140 days old and from the same brood but weighing 146 grams, the normal weight for this age. Each was raised under

identical conditions from the age of 38 days, except that rat 238 (see Chart LVI) was fed with a paste containing *casein* and protein-free milk, while in the food of 240 (see Chart CXIII) the casein was replaced by *gliadin*.

Plate 2 shows rat 220, E, fed on gliadin and protein-free milk but weighing only 58 grams, although 148 days old, and, for contrast, rat 168, D, of approximately the size normal for the age of rat 220, is also shown. Figure F shows a normally nourished rat of the same weight as rat 220. This picture is introduced to show that rat 220 has the appearance of a normal rat of corresponding size and weight. All these pictures were taken on exactly the same scale and afford a ready comparison of the relative sizes of the animals.

The interesting photographs of underfed cattle published by Waters, on the contrary, make the change of form in his undernourished animals of stationary weight quite apparent. We are, however, not prepared to assert that careful measurements of our stunted rats will not disclose some trace of similar changes in skeletal form. They must be slight at most; for we have often compared animals long maintained at small stature with properly grown animals which have just reached the same weight, without detecting any deviation from the youthful form in so far as one could judge by mere visual inspection. The photographs speak in the same sense.

The point on which we lay great stress in the foregoing experiments is the fact that the stunting is not attributable primarily to *under-feeding*. Our dwarfed rats have as a rule eaten as adequately as normally nourished animals *of the same size*. The energy factor, as such, thus drops out of the problem. In this respect the experiments are not comparable with those of Waters and of Aron, both of whom accomplished their results by underfeeding with adequate food materials. In our experiments the "energy requirement for maintenance" and the "energy requirement for growth," which together are essential to the developing organism, were both supplied. The rats did not grow primarily at the expense of stored tissue materials: they failed to grow in any sense. *We are obviously dealing with some other feature than insufficient energy supply*. The numerous illustrative experiments which will be cited later are accordingly to be interpreted as instances of *maintenance* without growth. If it is true that growth can only continue when the energy intake exceeds the mere maintenance requirement, it is equally true that an excess of calories does not *per se* insure growth in a suitable animal. Here then is the opportunity to ascertain and differentiate some of the essential qualitative factors: protein, inorganic salts, etc.—their minimum and optimum values.

EFFECT OF STUNTING ON THE GROWTH IMPULSE.

Before proceeding to study the influence of dietary variations on (*a*) maintenance and (*b*) growth, respectively, it became necessary to learn whether a more or less temporary inhibition of growth checks or in any degree modifies the capacity to grow (Wachstumstrieb). The literature on this subject by no means reveals a unanimity of opinion, although familiar experience will bring to mind many illustrations of compensated retardation of growth in children.* A few typical experiments may be cited. Rat 36 (male) kept stunted 49 days on a diet of gliadin food† (37 days) followed by casein food mixture† (12 days), showed complete recovery of growth on a mixed diet (see Chart XCVI). The "*mixed diet* of our animals consists of dog biscuit, sunflower seed, and fresh carrots (with occasional changes and addition of lumps of rock salt). Rat 65 (female) stunted, during 33 days on a diet of casein-zein food,‡ likewise resumed a normal rate of growth as soon as the mixed diet was instituted (see Chart XXXVII).

Special interest is attached to experiments in which after a preliminary stunting period the resumption of growth was accomplished on a diet containing milk as the effective component. Two protocols of the diet during the stunting period are reproduced in Table XXXV, with reference likewise to Charts XXVIII and XXIX.

TABLE XXXV.

Duration of stunting.	Rat 64 (female), 33 days.		Rat 51 (male), 46 days.	
		per cent		*per cent*
Stunting diet..........	Casein	12.0	Casein	18.0
	*Zein	6.0	Starch	29.5
	Starch	29.5	Sugar	15.0
	Sugar	15.0	Agar	5.0
	Agar	5.0	†Salt mixture I	2.5
	†Salt mixture I	2.5	Lard	30.0
	Lard	30.0		

*The zein was hydrated by the addition of a little water. †Cf., p. 86.

The curves in these cases are seen to be quite comparable with those of the normally growing rats. Bearing in mind that the animals here studied were continually kept in small cages under actual experimental conditions, the "normal" character of the growth curves makes it evident that the environment is no wise detrimental.

*Cf. Condereau: Recherches chimiques et physiologiques sur l'alimentation des enfants, Paris, 1869; Pagliani: Giornale della reale societa italiana d'igiene, Milano, 1879, I. (Quoted by Hatai: American Journal of Physiology, 1907, XVIII, p. 320.)

†See p. 122.

‡See p. 98. Water was added to this mixture until the zein was well hydrated.

Normal growth, as judged by curve of increase in body-weight, was resumed on a diet consisting of

	per cent.
"Trumilk"	60.0
Starch	16.7
Lard	23.3

Similar experiences are shown after feeding gliadin (Charts XCIX, C) or edestin (Chart LXV).

In the case of rat 37 (Chart XCVII), a stunting period of 49 days on a diet of gliadin food for 37 days, followed by casein food mixture for 12 days, was followed by normal resumption of growth under a dietary régime in which a period of feeding on the above milk-food was alternated with mixed food. Judging by the typical character of the curve of growth in this animal the two types of resuscitation diet, though radically different in origin, are equally efficacious in promoting growth. The growth curve shows little deviation from its usual course incidental to the changes in the dietary.

It may be remarked that the early stunting does not necessarily impair the capacity to breed at a later period when growth is again established. Furthermore, we have found that the milk-fat-starch mixture continued from early life in no wise impairs the potency of rats as breeders. Its nutritive efficiency will be referred to again.

Experiments such as those recorded above give unmistakable evidence of the fact that a considerable period of stunting by no means impairs the "Wachstumstrieb" of these animals. As soon as an appropriate diet is instituted growth begins anew and proceeds with practically the same speed as under normal conditions. By this we mean that a definite increment of gain from some fixed weight requires approximately the same period for its accomplishment as in the case of uninterrupted growth. A rat which will ordinarily grow from 60 grams to 180 grams in body-weight in 60 days will make the same gain even when its growth has been inhibited days or even weeks and its size and form retained at a maintenance level. This will be apparent by comparing, for example, the normal growth curve for both male and female rats with that of the realimented rats, during the same period of time, in Charts CXXII and CXXIII.

It should be emphasized that the situation is here quite different from that developed by Waters and Aron in the experiments on cattle and dogs. With their conditions of underfeeding the animals increase in size (height, etc.) while starving; and during the earlier period of such trials a poorly fed animal may actually gain in height as rapidly as a highly nourished one, fed to the limit of its appetite.*

*Cf. Waters: The capacity of animals to grow under adverse conditions. Proceedings Society for the Promotion of Agricultural Science, 1908, XXIX, p. 15.

The duration of the period of growth of the undernourished animal depends upon the constitutional vigor of the individual and the store of fat which it has accumulated. Quoting Aron: "Dem Einschmelzungsprozess fällt neben dem Fettgewebe in erster Linie die Muskulatur zum Opfer, während die Organe ihm widerstehen, wohl weil sie lebenswichtiger sind."

The results of realimentation in animals which show this "disproportionate" growth, *i. e.*, growth of one part at the expense of another, are not yet satisfactorily ascertained. Waters believes that physiological compensation may result "by an increase in the rate of growth in a period of liberal feeding following a period of low nourishment and low gain. In other words, an animal that is below the normal in size at a given age, through poor nourishment, apparently has the capacity, when liberally fed, to compensate for this loss, in a measure at least, by an increased rate of gain." He also suggests the possibility that growth may be accomplished on a more economical basis—a view which we are not yet ready to accept.

EFFECT OF PARTIAL STARVATION ON BODY-WEIGHT.

Hatai* has studied the effect of partial starvation followed by normal diet on the growth of white rats. The "partial starvation" consisted in feeding a diet that is practically devoid of protein, viz, starch and water, during 21 days to animals about 40 days old. The realimentation was continued to the age of maturity, at the end of 200 days. The statistics thus obtained and reproduced in Table XXXVI are presented graphically in Chart XXVI.

TABLE XXXVI.—HATAI'S MEASUREMENTS OF UNDERFED AND REALIMENTED RATS.

	Body-weight.			Total gain.	Ratio between initial and final.
	Initial.	After 21 days.	Final.		
	gm.	*gm.*	*gm.*	*gm.*	
Male, controls	35.2	63.1	224.4	189.2	1 : 6.37
Male, experimented	37.6	28.4	242.0	204.4	: 6.43
Female, controls	36.3	67.8	†172.6	136.3	: 4.75
Female, experimented	34.3	27.0	†167.8	133.5	: 4.89

Hatai concluded that, as far as body-weight is concerned, "the experimented rats have completely recovered from the effect of 21 days of partial starvation . . . The recovery in the weight is most astonishing, especially during the first 3 or 4 days, within which time the starved rats regain the weight lost during the 21 days of starvation. Later the increase in weight is very steady, though not as rapid as during the first few days, until the rat has reached the age

*Hatai: American Journal of Physiology, 1907, XVIII, p. 310.
†The body-weight in both control and experimented is small for the age.

of 150 days, and after this age increase in weight is relatively slow. What will happen to such rats during the later portions of the span of life has yet to be determined in order to answer the question whether this partial starvation in early life has any influence either on longevity or the onset of old age." (p. 314–315.)

EFFECT OF PARTIAL STARVATION ON NERVOUS SYSTEM.

Though the period of retarded growth was eventually completely compensated in Hatai's animals, in so far as the weight of the body and central nervous system are concerned, the chemical composition of the brain and spinal cord was not entirely free from the effect. As the result of an extended investigation of the effects of underfeeding on the nervous system, Donaldson* has arrived at the conclusion that one of the characteristics of growth, the change in the water content of the brain, has not been arrested like the increase of the animal in size and body-weight, but apparently accelerated. He states:

> The underfed group are in this character similar to somewhat older animals. Evidence further points to the continued formation of the medullary sheaths with advancing age even in rats which are underfed, *i. e.*, underfeeding does not arrest medullation. Underfeeding which stops growth of the body and retards that of the nervous system does not modify the percentage of water in the spinal cord, while it does reduce it in the brain—the amount of this reduction being less in the cases where the underfeeding is less severe.†

With respect to the possible psychological effects of such underfeeding and return to normal diet Donaldson says:

> So far as our tests show, such an experience does not modify the rat's ability to learn, for, by a series of experiments, it has been possible to determine that such a rat can learn to get its food under complicated conditions just as well and as rapidly as a normal animal (Hayes).‡

The preceding facts as to resuscitated rats are recorded here—despite the fact that this temporary stunting was produced by underfeeding (rather than unsuitable feeding as in our experiments)—because they suggest that the real story of the condition of the animals may perhaps not be revealed by the external evidences of growth. It is not at all impossible that the rats which we have dwarfed for months may have experienced some continued subtle changes in the make-up of the nervous system despite the appearance of unchanged youth which they manifest. Measurements of size and weight alone may not suffice to disclose the real physiological status of the animal, especially in respect to the development of the nervous functions and structures, which are singularly pro-

*Donaldson: Journal of Comparative Neurology, 1911, XXI, p. 139.
†Donaldson: *ibid.*, p. 169.
‡Donaldson: Journal of Nervous and Mental Disease, 1911, XXXVIII, p. 262.

tected even during starvation. This is seen to be true in the series of stunted animals fed on the glutenin mixture in our experiments (p. 72). There is a large field of investigation still open here with important bearings on the problems of retarded growth in man. According to Donaldson* "the progressive diminution of the percentage of water in the central nervous system with advancing age is to be regarded as an index of fundamental chemical processes, which take place in the more stable constituents of the nerve cells. These processes are but little modified by changes in the environment and taken all together constitute a series of reactions which express not only the intensity of the growth process in the nervous system, but also the span of life characteristic for any given species." Possibly, then, the further study of the nervous system in connection with our experiments may throw light on the phenomena of malnutrition (which our stunting experiments primarily represent) as well as those of undernutrition or starvation.

It may be well here to note that the experience of Donaldson† indicates the main features of human growth to be well represented in the albino rat. So good is the essential correspondence that there is every reason to continue the work on this form. The striking difference is that the rat grows some thirty times as rapidly as man.

COMPARISON OF MILK AND MIXED DIET.

The failure either to induce substantial growth in young rats or to satisfy completely the maintenance requirement of older animals during very long protracted periods on the mixtures of isolated foodstuffs thus far reported raises the question as to what constitutes an ideal nutriment for a rat. The suitability of mixed diet is beyond question. The favorable experiences with dried milk powder (some of which have been recorded on pages 75 and 76) early directed our attention to this product. Rats were not only resuscitated after nutritive decline and suitably maintained, but also grown from early age on pastes in which the milk powder (with lard and starch) constituted the mixture. The commercial brand "Trumilk"‡ employed by us has been analyzed at the Connecticut Agricultural Experiment Station with the following results:

	Per cent.	
Water		3.8
Total solids		96.2
Protein (N×6.38)	25.6	
Fat	27.4	
Lactose	37.2	
Ash	6.0	

*Donaldson: Journal of Comparative Neurology, 1910, xx, p. 143.

†Cf. Donaldson: Journal of Nervous and Mental Disease, 1911, xxxvii, p. 258.

‡This product was kindly furnished to us in powder form by the Merrell-Soule Co., Syracuse, N. Y.

The preparation apparently contains a small excess of iron over that found in cow's milk—probably as a contamination from the desiccating process used. It is obtainable in easily manipulated form and with the addition of a small amount of nitrogen-free lard and starch forms a food paste readily consumed by rats. These pastes have been used, either with or without our earlier standard salt mixture (I),* as follows:

	Per cent.	Per cent.
"Trumilk"	60.0	60.0
Starch	16.7	15.7
Lard	23.3	23.3
Salt mixture I	0.0	1.0
	100.0	100.0
Nitrogen content	2.5	2.5

We have carried rats through the period of growth as well as pregnancy on this diet alone, from the time that they were removed from the mother (cf. Charts XXXI, XXXII, and XXXIII).

As a further illustration of the excellent nutritive properties and physiologically appropriate "combination" of food ingredients in the milk food-mixture, illustrative charts are appended to show the recovery of rats moribund after prolonged periods of malnutrition, with lack of inorganic salts in the dietary (Charts XXXIV and XXXV). Many similar illustrations might be reproduced, giving evidence of the perfect realimentation of rats by the use of the milk food (cf. Charts XXVIII, LXV, XCIX, and C).

Remembering that our earlier trials with casein, the chief protein ingredient of the milk powder, and with combinations of casein and other proteins were at best successful only in maintaining nutritive equilibrium—and that not indefinitely—and were never adequate for the manifestation of real growth, we directed our attention to the non-protein constituents of milk. After numerous failures to modify the inorganic and non-protein ingredients of our dietaries by altering the relation of proportions of the various ions as well as the character of the carbohydrates and fats, it occurred to us that the protein-free portion of the milk might give the clue to the successful feeding of proteins which did not appear to be the inefficient factors in our cases of malnutrition. Accordingly a product was prepared as follows:

Perfectly fresh centrifugated milk, nearly free from fat, was precipitated in lots of about 36 liters by diluting with 7 liters of distilled

*This mixture, prepared in imitation of Röhmann's successful product and empirically found by use to be the most satisfactory of the different combinations tried, has the following composition:

	Grams.		Grams.
$Ca_3(PO_4)_2$	10.0	Mg citrate	8.0
K_2HPO_4	37.0	Ca lactate	8.0
NaCl	20.0	Fe citrate	2.0
Na citrate	15.0		100.0

(Cf. our previous report, Feeding experiments with isolated food-substances, Publication No. 156, Carnegie Institution of Washington, p. 32.)

water which contained 1.64 c.c. of concentrated hydrochloric acid. The flocculent precipitate of casein was strained out on cheesecloth and the very nearly clear solution was filtered through a pulp filter. The filtrate, which at the most was very slightly turbid from suspended fat, was tested carefully by the alternate addition of dilute alkali and acid to determine whether any more casein could be separated from it. The addition of alkali caused a slight precipitate which did not increase on adding more alkali or dissolve on the addition of even relatively large amounts of alkali. This was presumably chiefly calcium phosphate. The addition of acid in no case caused any further precipitation. The filtered milk serum was then heated to boiling for a few minutes and filtered. The filtrate, which was in all cases water clear, was then neutralized to litmus with a dilute solution of sodium hydroxide and evaporated to dryness on a steam bath at a temperature of about 70°. The product thus obtained formed a friable, pale yellow mass which was easily reduced to a fine powder by grinding in a mill. Several grams of this powder were tested for protein by dissolving in about 30 c.c. of water containing a little hydrochloric acid and warming gently. The solution was then saturated with ammonium sulphate. The precipitate, which appeared to consist chiefly of calcium sulphate, was separated by centrifugation, dissolved in a little water, and potassium hydrate solution and copper sulphate added. The solution showed no evidence of the biuret reaction until it was saturated with potassium hydroxide and shaken with alcohol. It then separated into two layers, the upper alcoholic layer showing a slight but positive biuret reaction. Millon's reaction tried on portions of 2 or 3 grams of the substance did not give a positive reaction. Nitrogen determinations in several lots of the protein-free milk powder thus made showed them to contain 0.66, 0.59, 0.60, 0.72, 0.71, 0.67, 0.75 per cent of nitrogen. Munk* states that if the proteins of milk are precipitated by alcohol, or separated according to Hoppe-Seyler, from one-thirtieth to one-fifteenth of the protein remains dissolved. All the proteins can be precipitated only by tannin in the cold or by copper hydroxide on heating. He further states that cow's milk contains about one-sixteenth of its nitrogen in non-protein form. Since our protein-free milk powder was equal to 50 per cent of the total solids of the milk, it should, if Munk's statements are correct, contain 0.48 per cent of non-protein nitrogen, thus leaving at the most only 0.28 per cent of protein nitrogen, equal to 1.69 per cent of protein. Since 100 grams of the food mixture employed in our experiments contained 28.2 grams of protein-free milk powder, we can assume that at the most the food pastes thus made contained only 0.48 per cent of milk protein. The protein-free

*Munk: Virchow's Archiv für pathologische Anatomie, 1893, 134, p. 501.

milk powder thus produced as above described left about 14.5 per cent of inorganic matter on ignition. This includes not only the inorganic constituents of the milk, although by no means in the combination in which they occur in the mammary secretion, but also the inorganic salts which were formed by the addition of the hydrochloric acid used to precipitate the casein and also the sodium salts which resulted from neutralizing the milk serum with sodium hydroxide solution.

EXPERIMENTS WITH ISOLATED PROTEINS AND "PROTEIN-FREE" MILK.

The use of this product (which we shall designate as protein-free milk) as an adjuvant to isolated proteins to furnish the inorganic elements of the diet has succeeded beyond our expectation. When employed, for example, in combination with various proteins, in the proportion in which its ingredients occur in the complete milk food already used (see page 76), it induces normal growth. Added during the periods of nutritive decline to food mixtures which no longer suffice to maintain rats, recovery has manifested itself in practically every case. Where, as in the case of zein, gliadin, or hordein feeding, no advantage has been obtained by the use of the protein-free milk, it has become obvious that the protein *per se* is the defective food constituent. Thus at length we have found a method of controlling or furnishing some of the most essential non-protein factors in the diet, so that the value of the individual proteins can be investigated under much more favorable conditions than formerly.

Numerous charts (see p. 103 fig.) present the graphic records of feeding experiments with casein, edestin,* glutenin,* glycinin,* gliadin,* hordein,* ovalbumin,† and lactalbumin,‡ showing appropriate growth, or maintenance, according to the age at which the animals were started on the use of the protein-free milk as the non-protein component in place of the earlier inorganic salt mixture.

It might be objected, after superficial consideration of these results, that the favorable outcome (especially for growth) is due to milk protein contaminating the "protein-free milk" component of the diet. Aside from the fact that the amount of possible contamination is at most small, evidence of the untenability of such a theory is available from several sources. In the first place, growth has not followed the use of *all* proteins when the protein-free milk was added to them.

*For the preparation of these vegetable proteins see T. B. Osborne: Darstellung der Proteine der Pflanzenwelt, Abderhalden's Handbuch der biochemischen Arbeitsmethoden, 1909, II, p. 270.

†This was prepared by Hopkins's method and was free from conalbumin. Cf. Osborne, Jones, and Leavenworth: American Journal of Physiology, 1909, XXIV, p. 252.

‡The preparation of this is described on p. 81.

The results can be grouped in two series, viz:

Diet = Isolated protein, protein-free milk, starch, agar, fat.

Group I.—Young rats.	Group II.—Young rats.
Active growth with— Casein (Charts XLVI, XLVII, LII, LIII, LIV, LV, LVI, LVII, LVIII, LIX, and LX. Ovalbumin (Charts XC and XCI). Lactalbumin (Charts XCII and XCIII). Edestin (Charts LXXI, LXXII, LXXIII, LXXIV, LXXV, and LXXVI). Glutenin (Charts LXXXVII, LXXXVIII, and LXXXIX). Glycinin (Charts XCIV and XCV).	Little or no growth with— Gliadin (Charts CVIII, CIX, CX, CXI, CXII, CXIII and CXIV). Hordein (Charts CXXIV and CXXV).

The failures in group II lead to the conclusion that the proteins, gliadin and hordein, are inadequate for the functions of growth. We are presumably dealing with a chemical inadequacy rather than any toxicity and consequent lack of growth, judging by the fact that the gliadin and hordein rats are maintained in good, nutritive condition even in the absence of growth. Their body-weight is scarcely changed at all. Without the use of the protein-free milk or fæces-feeding gliadin rats have usually declined (Charts XCVIII, XCIX, and C).

A second reason why the success of these trials is not due to the presence of possible minute contaminations with milk protein is discoverable in Charts XLIII, XLIV, XLV, XLVIII, XLIX, L, LI, CVIII, CIX, CX, and CXI. Here the addition of not inconsiderable portions (5 to 30 per cent) of the actual milk food to the earlier inefficient protein mixtures is incapable of bringing about growth in any degree equal to that at once initiated when the protein-free milk is added in relative abundance.

Further evidence that a trace of milk proteins is not responsible for the growth of the rats fed with mixtures containing our protein-free milk powder is furnished by experiments in which successively larger quantities of the milk food are added to the gliadin food. Here we see that growth gradually increases with the larger additions of the milk food, although with even as much as 30 per cent in the food the rate of growth is much below normal. With additions of 5 or even 20 per cent of the milk food, the rate of growth is very slow, as shown by Charts CIV, CV, CVI, and CVII. That this result is to be attributed to the proteins introduced in the milk food and not to a combination of a small quantity of milk proteins together with a sufficient quantity of the inorganic or other constituents of the milk is shown by experiments now in progress in which the addition of the milk food to the gliadin and protein-free milk food is producing normal growth. In this mixture we have all of the constituents of

the protein-free milk present in the same proportions as in our milk food, but less than one-third of the protein constituents of the milk. It is therefore evident that only a small proportion of the protein constituents of the milk are required to produce normal growth, and it may be assumed that the presence of a small quantity of milk proteins in our protein-free milk powder would manifest itself by at least some slight growth.

DISCUSSION OF THE RESULTS AND THEIR BEARINGS.

We have stated that by our plan a biological comparison of different proteins in respect to their rôle in growth can at length be made. Our work in this direction must be regarded as barely begun. Nevertheless it is of interest to speculate as to the indications already gained and the outlook for future work. A comparison of the two groups of proteins—those adequate and those inadequate for growth purposes—at once reveals the fact that the latter category comprises proteins (gliadin, hordein, zein) commonly spoken of as chemically "incomplete." They lack one or more of the amino-acid complexes which are obtainable from the so-called "complete" proteins. None of them furnish glycocoll or lysine, and zein in addition is devoid of tryptophane. By feeding relatively small quantities of proteins like casein with gliadin growth begins at once. Here we can determine the minimum of suitable protein to satisfy this growth requirement—a study already begun (cf. Charts CXX, CXXI, CXXII, and CXXIII). The addition of amino-acids to "complete," as it were, the inadequate proteins can now be studied amid controllable factors; the biological rôle of hydrolyzed proteins and the significance of complete hydrolysis or digestion in nutrition can be examined anew.

The experiences which have demonstrated the striking differences in value of the individual proteins and the small proportion of casein which suffices to induce growth instead of standstill (cf. Charts CXX, CXI, CXXII, and CXXIII, for example) emphasize the importance of the *purity* of the protein fed. We have devoted much labor and incurred a very considerable expense to obtain proteins in a form as uncontaminated as present methods will permit. The products used were as pure as one would expect them to be if employed for purposes of refined protein analysis. Had less perfect products been employed it is quite conceivable and indeed likely that the admixtures would have sufficed to alter completely the outcome of many experiments. For example, gliadin is prepared free from glutenin only be very careful purification methods; and although the nutritive properties of these two companion proteins are extremely unlike, as clearly indicated by our trials, a failure to effect a complete separation of a little glutenin from gliadin would have been sufficient to prevent the deficiencies of the latter from exhibiting themselves. Or

again, failure to purify carefully a protein like casein will vitiate the study of a problem like the synthesis of amino-acids. Pure casein is glycocoll-free; and the continued feeding of such a product as the sole protein of the dietary enables one to make deductions respecting the synthesis of glycocoll. The use of crude commercial protein preparations can never satisfy the requirements of refined study in this domain, where small effects continued over long periods are of great importance. We believe, therefore, that such considerations justify the energy and expense which have been put into the work.

In relation to the much-discussed problem of the relative value of organic *vs.* inorganic phosphorus in nutrition, our data after feeding phosphorus-free edestin to growing rats (cf. Charts LXXV and LXXVI) show a success quite as great as that with phosphorus-containing casein (cf. Charts LVI, LVII, LVIII, LIX, and LX). The animals must here have synthesized their phosphorus compounds from inorganic phosphorus. Whether milk production and other functions calling for such synthetic reactions will continue adequately is open to investigation. It is also noteworthy that all of our animals grow on a dietary that is purine-free, or essentially so. Here the question of purine synthesis suggests itself. It is apparent, *e. g.*, in the case of gliadin, that the grown as well as ungrown rats may be *maintained* for long periods on single proteins.

With such an ideal non-protein dietary component at hand amino-acid substitutions can be attempted in the adult as well as in the growing animal. The protein minimum (or minima) is also open to accurate investigation. With a method of feeding devised which will permit a differentiation between growth and maintenance, which furnishes an energy-yielding protein-free component that is appropriate, and leaves the protein as the sole variable in the dietary, we believe that further contributions can be made to the problems of nutrition.

In the preparation of the large quantities of carefully purified proteins required for these experiments, we have been assisted by Mr. Charles S. Leavenworth, Mr. Owen Nolan, Mr. Leigh I. Holdredge, and Mr. Lawrence Nolan, whose valuable cooperation we are glad to acknowledge here.

THE CHARTS AND THEIR EXPLANATIONS.

In the following charts, to which reference is made in various places in the text, the abscissæ of the curves represent days and the ordinates actual body-weight (solid line) or food-intake (dotted line) in grams. In some of the charts the average (normal) curve of growth, plotted from body-weight data available for normally growing animals of the same sex, is represented by a broken line for comparison. The food-intake curve is plotted from the quantities of food eaten per week. The numbers on the body-weight curves indicate the time at which changes in the character of the feeding were instituted. All curves in this paper are plotted on the same scale, so that they are directly comparable.

Salt mixture I, to which reference is frequently made, was composed of—

	Grams.
$Ca_3 (PO_4)_2$	10.0
K_2HPO_4	37.0
NaCl	20.0
Na citrate	15.0
Mg citrate	8.0
Ca lactate	8.0
Fe citrate	2.0
	100.0

INDEX OF CHARTS WITH REFERENCE TO FOOD-MIXTURES AND PROTEINS FED.

[Numbers refer to pages in the text.]

CHART XXII.

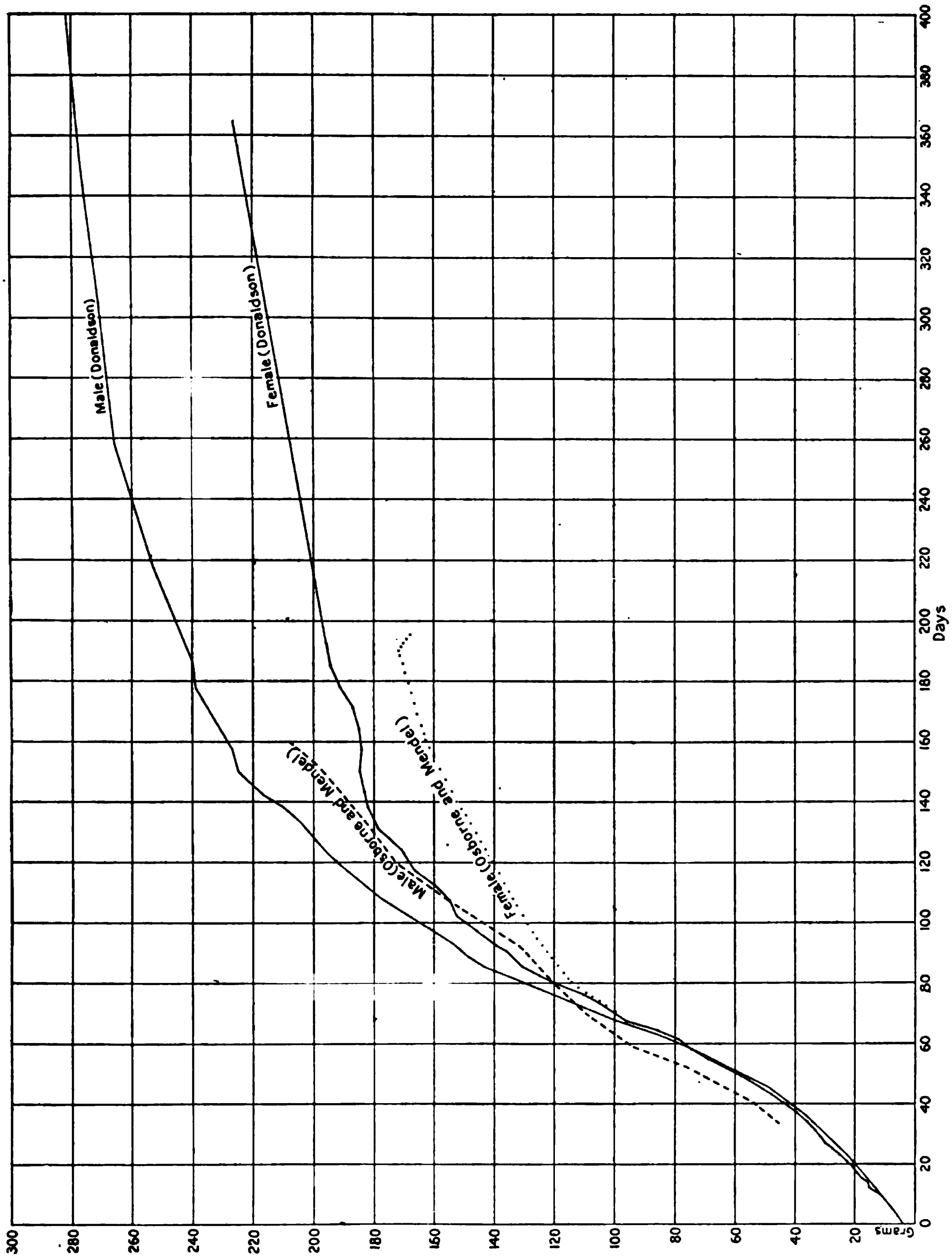
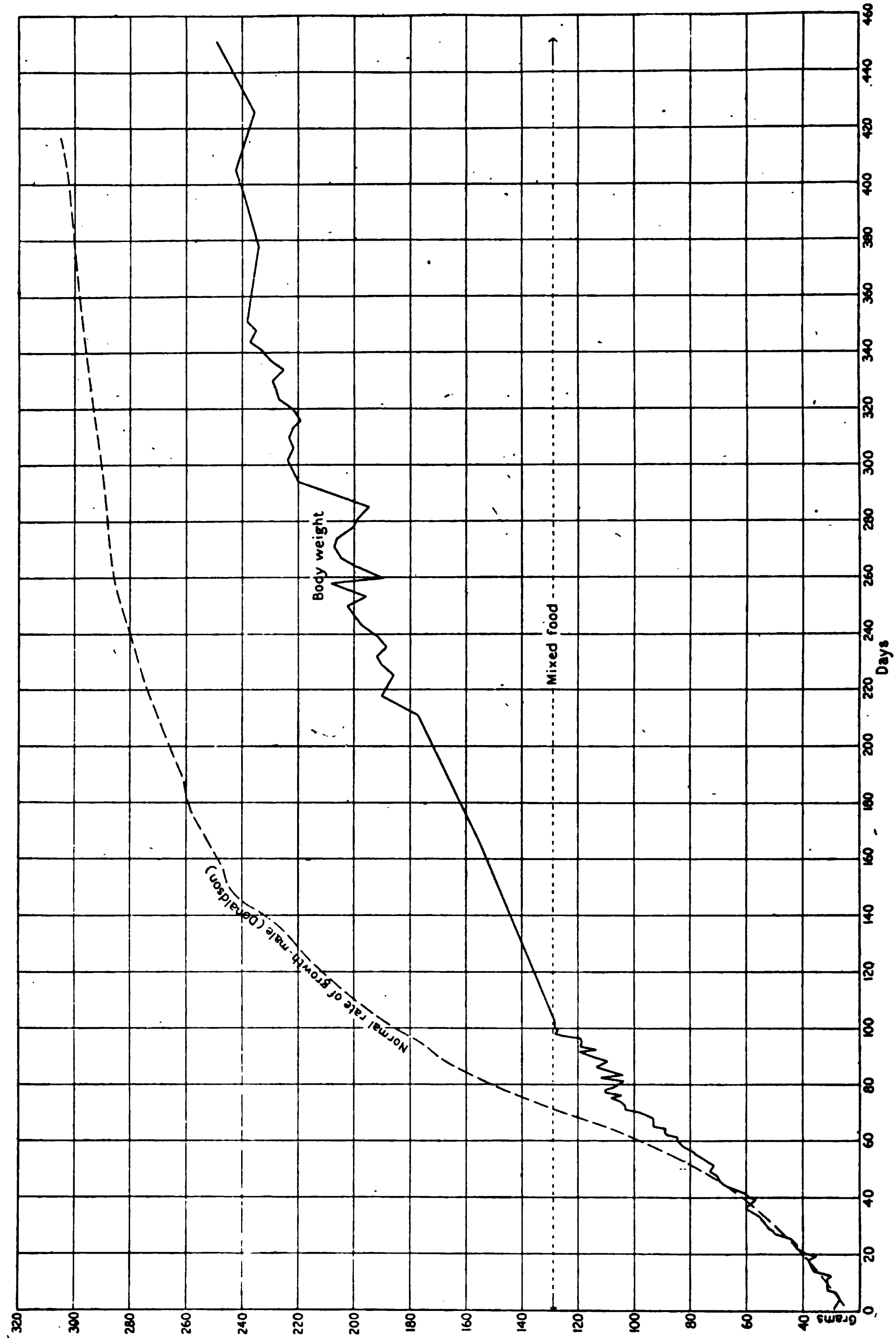


Chart XXII shows average normal rates of growth of male and female white rats according to Donaldson and to Osborne and Mendel. In our experience the female rat does not attain as large a size as in Donaldson's experiments. The growth curves coincide until the animals reach an age of about 70 days.

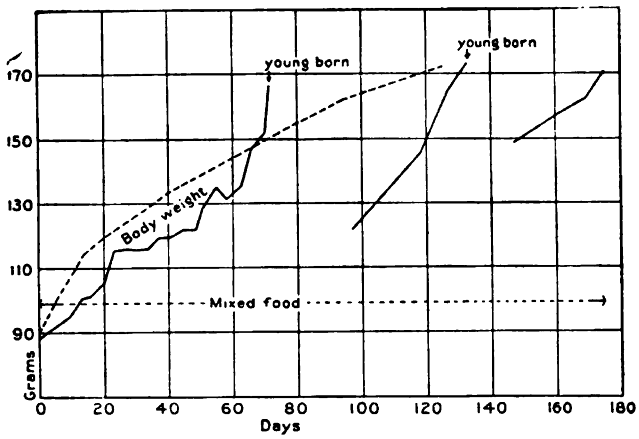
CHART XXIII.



Body weight
Mixed food
Normal rate of growth-male (Donaldson)
Days
Grams
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460

Chart XXIII (rat 48, male) shows the growth of the male rat from early life, under cage conditions adopted for experimental feeding. The animal was fed 452 days on mixed food, consisting of dog-biscuit, sunflower and other seeds, fresh vegetables, and salt.

CHART XXIV.



Charts XXIV (rat 166, female) and XXV (rat 156, female) show the typical growth of female rats, including pregnancy, under cage conditions. The animals were fed on mixed food.

CHART XXV.

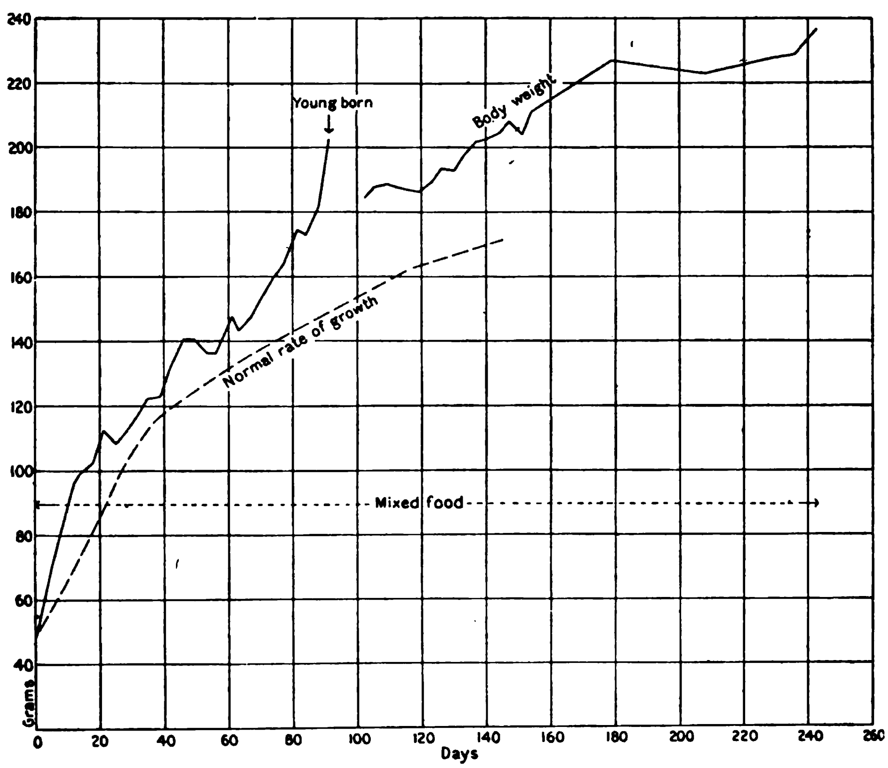


CHART XXVI.

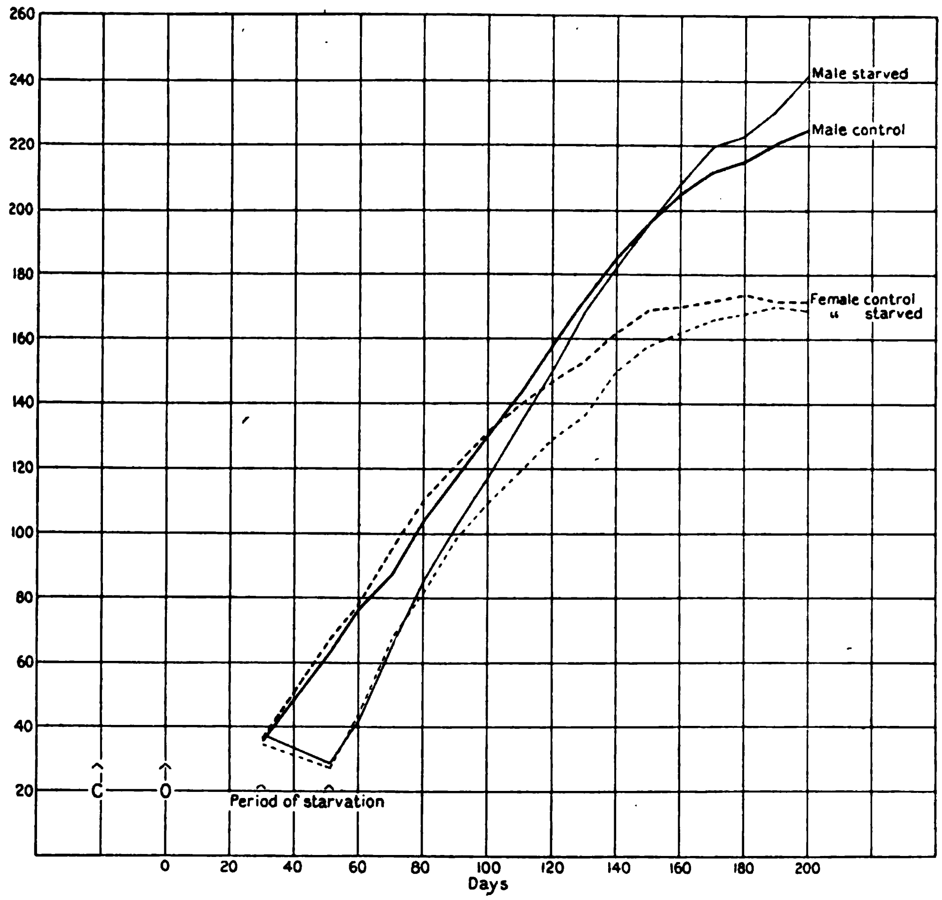


Chart XXVI (curves, from Hatai, American Journal of Physiology, 1907, XVIII, p. 311) shows the body-weights of albino rats at different ages. *C*, conception, and *O*, the date of birth 21 days after conception. An illustration is given of the influence on growth of a period of 21 days of starvation during early life.

CHART XXVII.

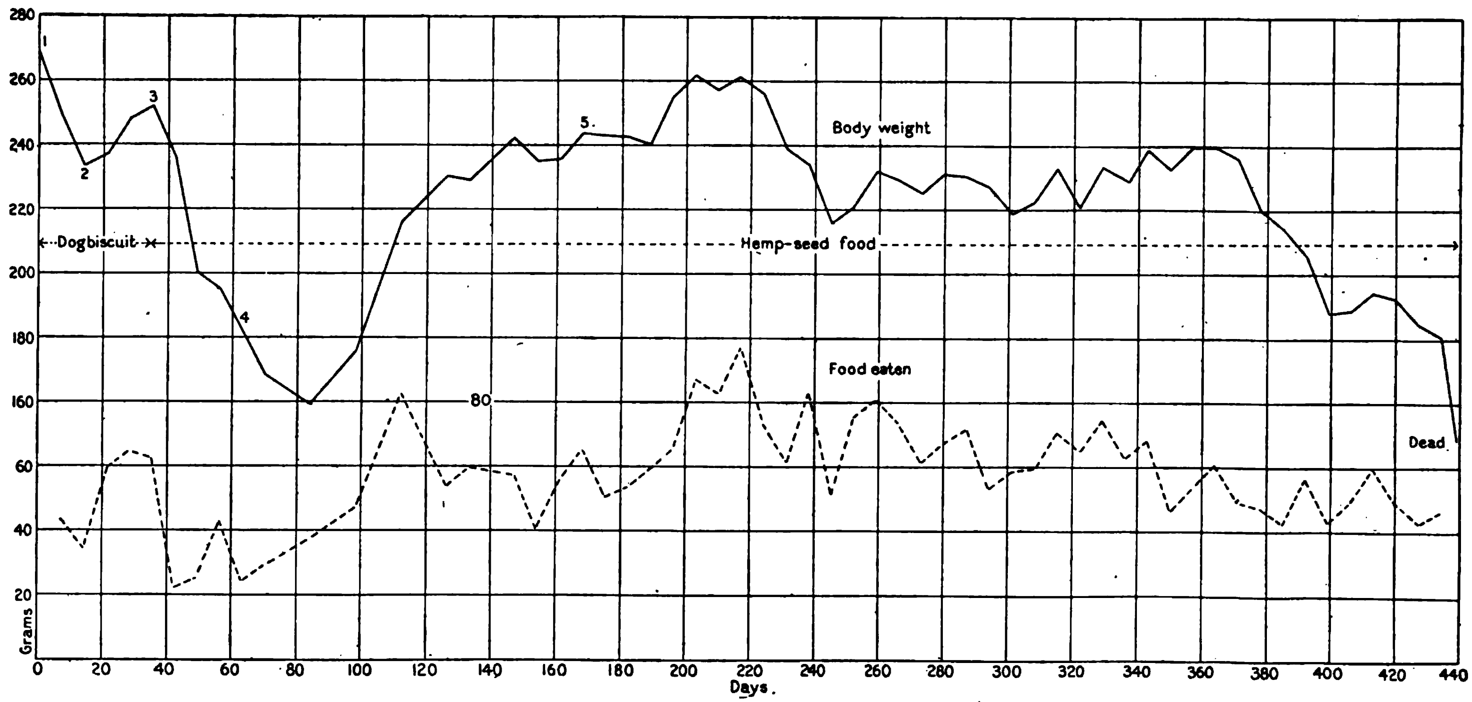


Chart XXVII (rat 18, male) shows the possibility of feeding the same food-mixture (hempseed paste) for a very long period. The diets of this animal for 5 periods were as shown in the table. The hempseed paste was fed for 404 days. Other data will be found on pp. 21–23 in Publication 156, Part I, of the Carnegie Institution of Washington. The food intake was nearly uniform and adequate until near the end of the animal's life. Death was caused by pulmonary disease.

Constituents.	Per. 1.	Per. 2.	Per. 3.	Per. 4.	Per. 5.
	p. ct.	p. ct.	p. ct.	p. ct.	p. ct.
Dog biscuit....	58.4	70.0	0.0	0.0	0.0
Lard..........	41.6	30.0	10.0	10.0	10.0
Hempseed.....	0.0	0.0	46.0	46.0	50.0
Starch........	0.0	0.0	42.0	42.0	38.0
NaCl.........	0.0	0.0	2.0	0.0	0.0
Salt mixture I.	0.0	0.0	0.0	2.0	2.0

CHART XXVIII.

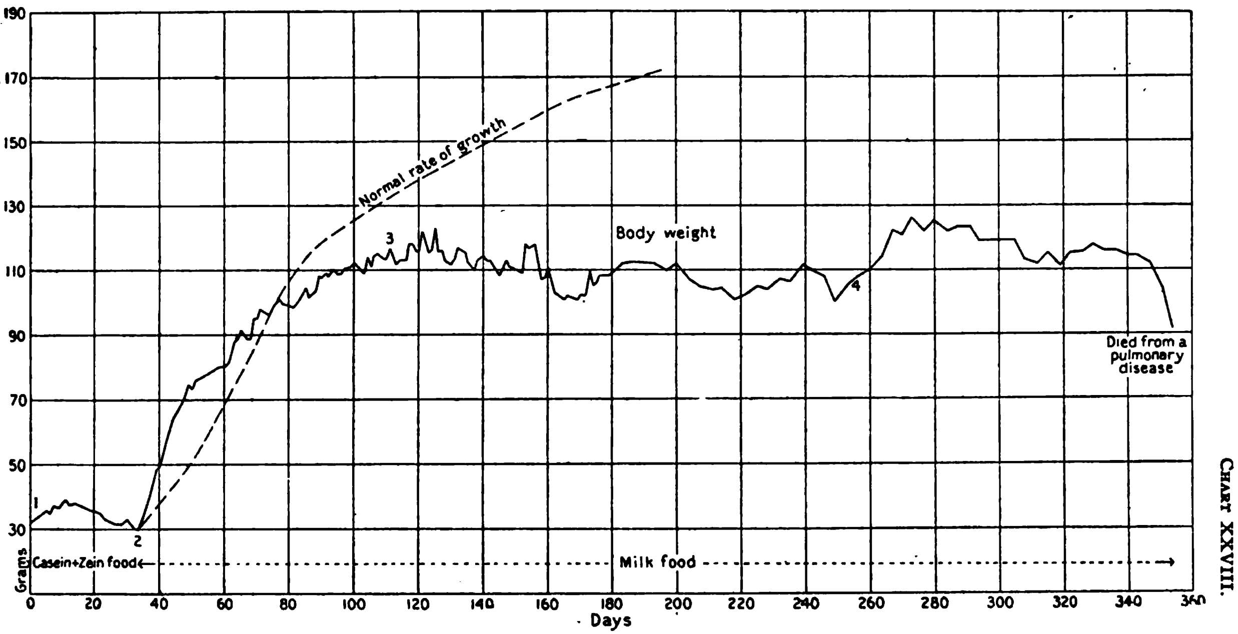


Chart XXVIII (rat 64, female) shows a period of stunting during 33 days in early life, followed by resumption of growth and maintenance on a milk diet for more than 300 days. The animal died of an intercurrent disease. The diets during the several periods were as shown in the table. Observe that the essential change during the long period of milk-paste feeding consisted in modifying the inorganic constituents of the food; also note the entire absence of roughage or agar in the diet after period 1.

Constituents.	Per. 1.	Constituents.	Periods 2 and 4.	Per. 3.
	p. ct.		p. ct.	p. ct.
Casein	12.0	Trumilk	60.0	60.0
Zein	6.0	Starch	16.7	15.7
Starch	29.5	Salt mixture I	0.0	1.0
Sugar	15.0	Lard	23.3	23.3
Agar	5.0			
Salt mixture I	2.5			
Lard	30.0			

CHART XXIX.

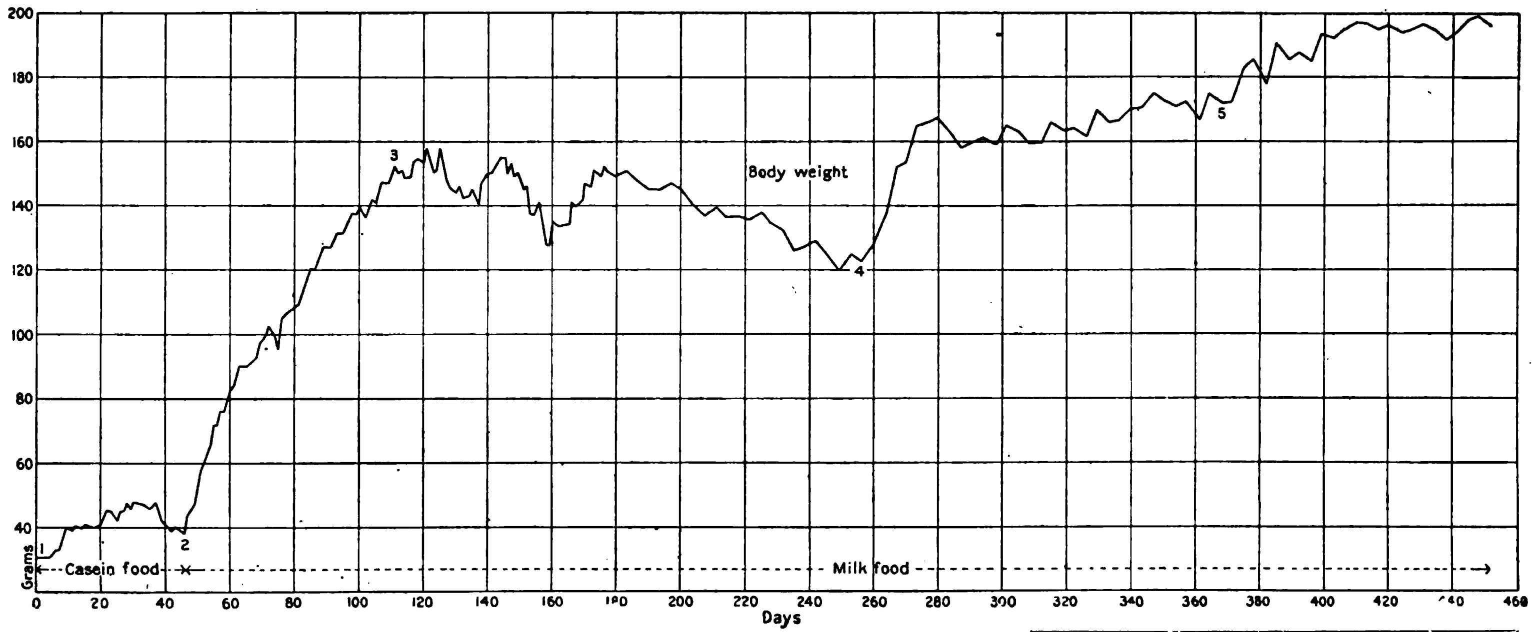


Chart XXIX (rat 51, male) shows early stunted growth on casein (46 days) and recovery on milk diet (406 days). The influence of changes in inorganic salts on nutritive condition is also shown in periods 2 and 4 contrasted with periods 3 and 5. Note the entire absence of roughage or agar in the diet after period 1. The diets were as shown in the table. The efficiency of the milk-paste long continued as a food is apparent, as well as the maintenance of the rat in good health under cage conditions and on a "monotonous" diet.

Constituents.	Per. 1.	Constituents.	Periods 2 and 4.	Periods 3 and 5.
	p. ct.		*p. ct.*	*p. ct.*
Casein.........	18.0	Trumilk.......	60.0	60.0
Starch.........	29.5	Starch.........	16.7	15.7
Sugar.........	15.0	Salt mixture I.	0.0	1.0
Agar.........	5.0	Lard..........	23.3	23.3
Salt mixture I.	2.5			
Lard..........	30.0			

CHART XXX.

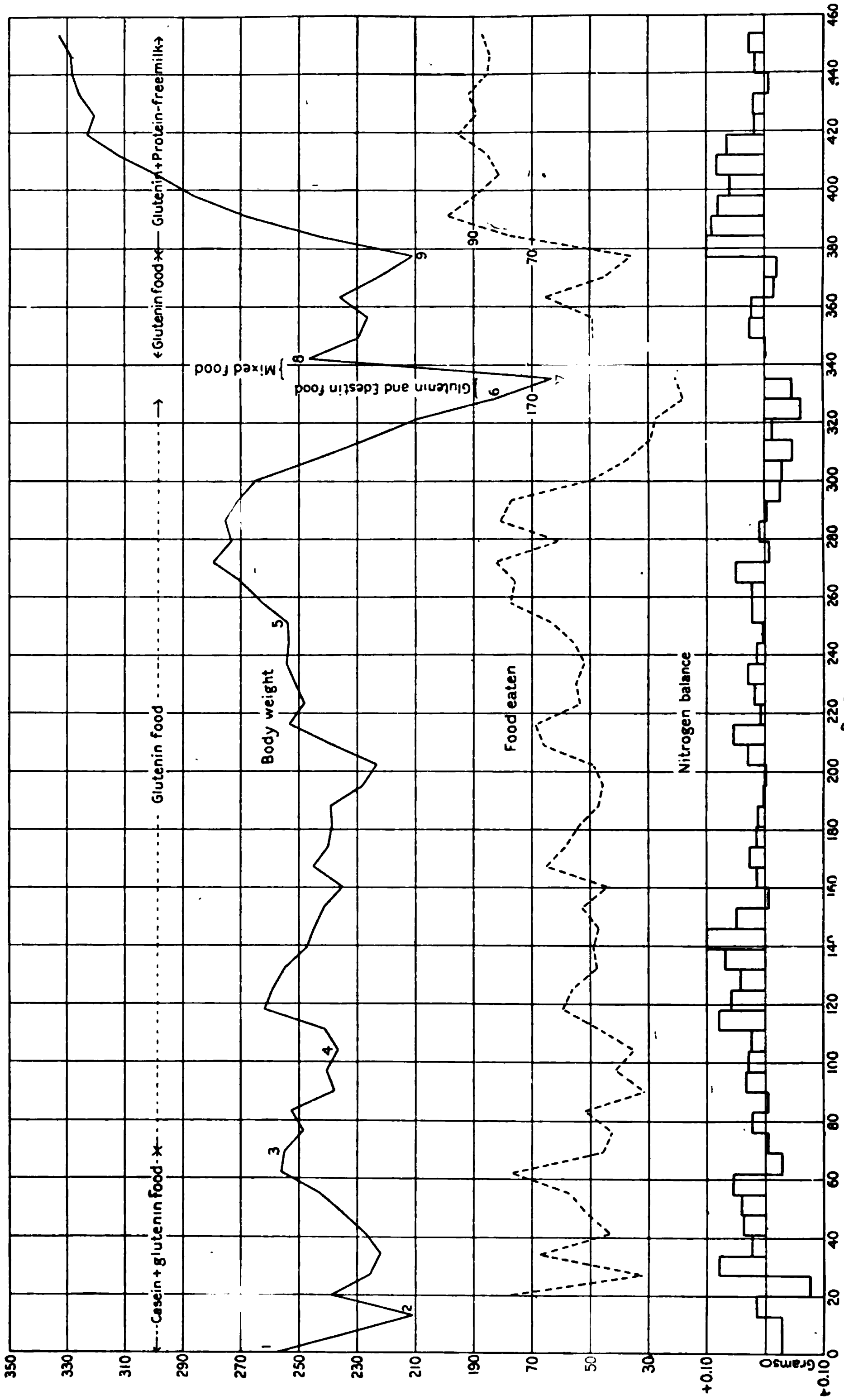

Casein + glutenin food
Glutenin food
Glutenin food
Glutenin + Protein-free milk
Mixed food
Glutenin and Edestin food
Body weight
Food eaten
Nitrogen balance
Days
Grams

Chart XXX (rat 71, male) shows long-continued feeding of isolated foodstuffs and also long-continued maintenance on glutenin from wheat as the only protein. The history of the animal is on p. 59. The diets were as follows:

Constituents.	Per. 1.	Per. 2.	Per. 3.	Per. 4.	Periods 5 and 8.
	p. ct.	*p. ct.*	*p. ct.*	*p. ct.*	*p. ct.*
Glutenin	6.0	6.0	16.36	18.0	18.0
Casein	12.0	12.0	0.0	0.0	0.0
Starch	29.5	24.5	22.27	14.5	34.5
Sugar	15.0	15.0	13.63	15.0	20.0
Agar	5.0	5.0	4.54	5.0	5.0
Salt mixture I	2.5	2.5	2.27	2.5	2.5
Lard	30.0	35.0	40.93	45.0	20.0

Constituents.	Per. 6.	Per. 7.	Constituents.	Per. 9.
	p. ct.			*p. ct.*
Glutenin	9.0	Mixed food.	Glutenin	18.0
Edestin	9.0		Protein-free milk	28.2
Starch	33.5		Starch	23.8
Sugar	18.5		Agar	5.0
Agar	5.0		Lard	25.0
Salt mixture I	2.5			
Lard	23.5			

Chart XXX further shows the possibility of maintaining an animal satisfactorily under our cage conditions for 458 days. Attention is particularly directed to period 9, during which the only change in the diet consisted in substituting protein-free milk for some of the non-protein components of the dietary. The lowest line represents the nitrogen balance of the rat.

CHART XXXI.

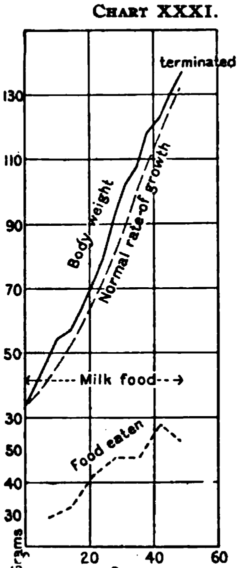


CHART XXXII.

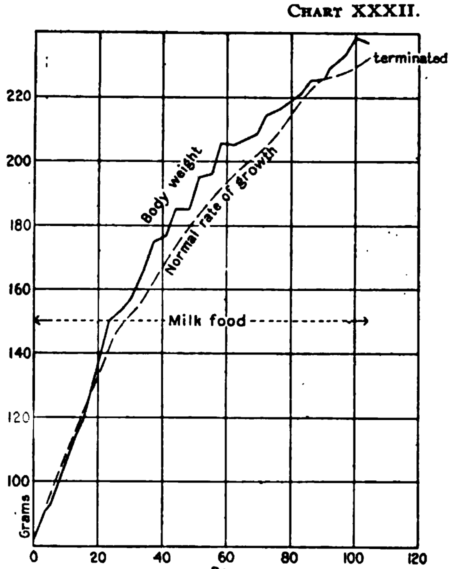


Chart XXXI (rat 222, male) shows early growth curve of male on milk diet, having the following composition: Trumilk, 60.0 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1.0 p. ct.; lard, 23.3 p. ct.

Chart XXXII (rat 195, male) shows normal growth curve of male on milk diet, having the following composition: Trumilk, 60 p. ct.; starch, 16.7 p. ct.; lard, 23.3 p. ct.

CHART XXXIII.

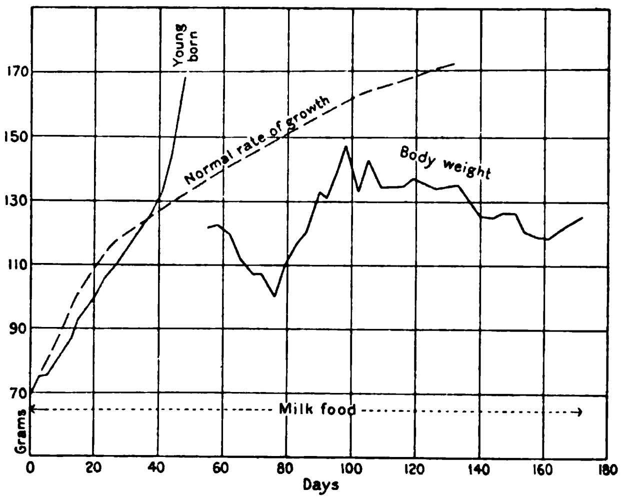


Chart XXXIII (rat 181, female) shows growth and normal pregnancy of female on milk food, consisting of Trumilk, 60 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1.0 p. ct.; lard, 23.3 p. ct.

CHART XXXIV.

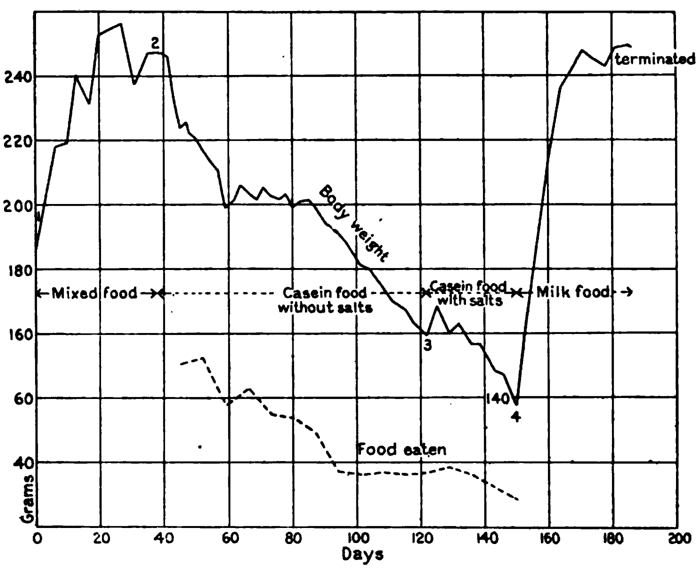


Chart XXXIV (rat 106, male) shows malnutrition induced by lack of inorganic salts in the dietary and subsequent perfect recovery on milk-paste. The diet was mixed food for period 1; for the remaining periods as follows:

Constituents.	Per. 2.	Per. 3.	Constituents.	Per. 4.
	p. ct.	*p. ct.*		*p. ct.*
Casein	18	18.0	Trumilk	60.0
Starch	25 to 32.5	32.5	Starch	15.7
Sugar	17 29.5	21.9	Salt mixture I	1.0
Agar	0 5.0	0.0	Lard	23.3
Salt mixture I	0 0.0	2.6		
Lard	20 35.0	25.0		

CHART XXXV.

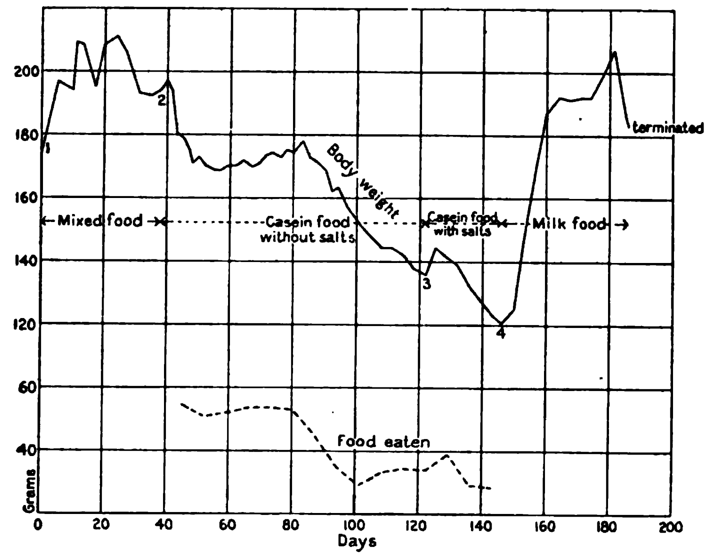


Chart XXXV (rat 110, female) shows malnutrition induced by lack of inorganic salts in the dietary and subsequent perfect recovery on milk-paste. The diet consisted of mixed food for period 1, and as follows for the remaining periods:

Constituents.	Per. 2.	Per. 3.	Constituents.	Per. 4.
	p. ct.	*p. ct.*		*p. ct.*
Casein	18	18.0	Trumilk	60.0
Starch	25 to 32.5	32.5	Starch	15.7
Sugar	17 29.5	21.9	Salt mixture I	1.0
Agar	0 5.0	0.0	Lard	23.3
Salt mixture I	0	2.6		
Lard	20 35.0	25.0		

CHART XXXVI.

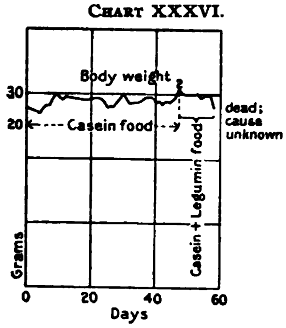


Chart XXXVI (rat 54, male) shows the maintenance for 46 days of a very small rat, without growth, on a diet in which casein formed the sole protein. The composition of the food was as shown herewith:

Constituents.	Per. 1.	Per. 2.
	p. ct.	*p. ct.*
Casein	18.0	9.0
Pea legumin	0.0	9.0
Starch	29.5	29.5
Sugar	15.0	15.0
Agar	5.0	5.0
Salt mixture I	2.5	2.5
Lard	30.0	30.0

CHART XXXVII.

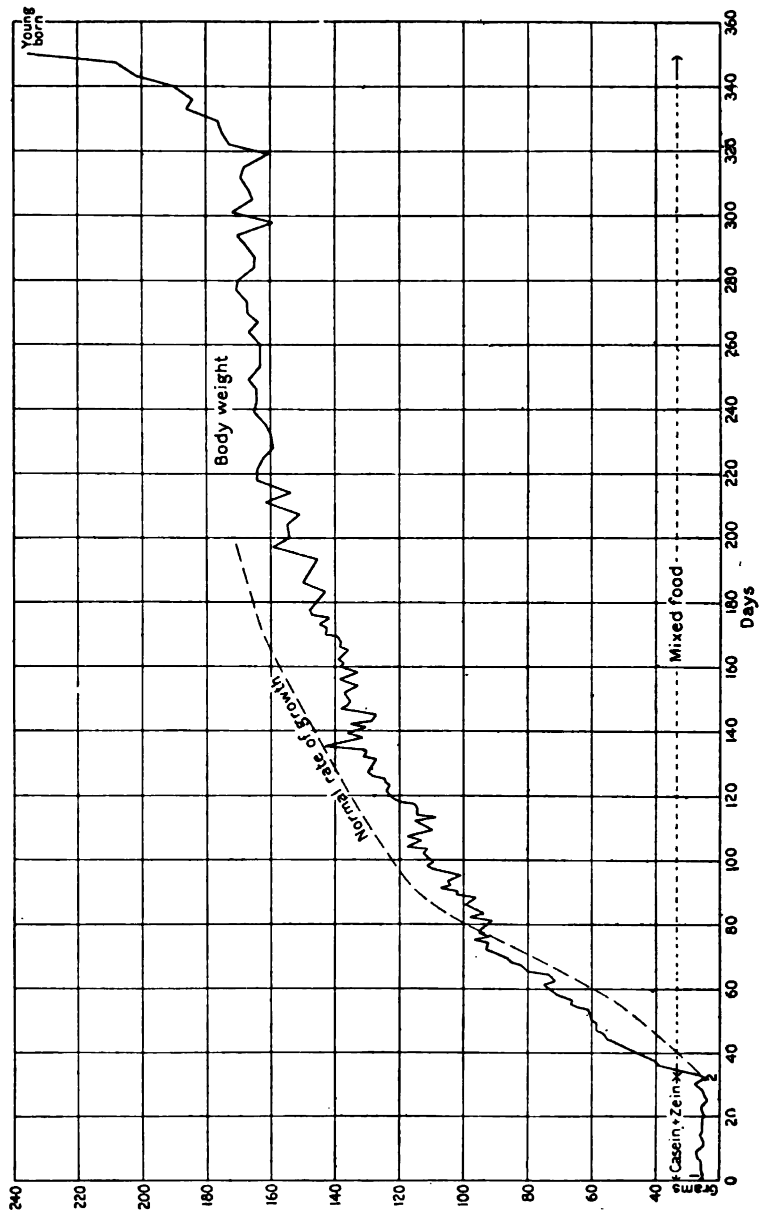


Chart XXXVII (rat 65, female) shows stunting for 33 days during early life, followed by normal growth and pregnancy on mixed food. In addition to the typical growth during 317 days, the curve emphasizes the unaltered "capacity to grow" after stunting by improper diet. The diet during period 1 was as shown herewith.

	p. ct.
Casein........	12.0
Zein..........	6.0
Starch........	29.5
Sugar.........	15.0
Agar..........	5.0
Salt mixture I.	2.5
Lard..........	30.0

CHART XXXVIII.

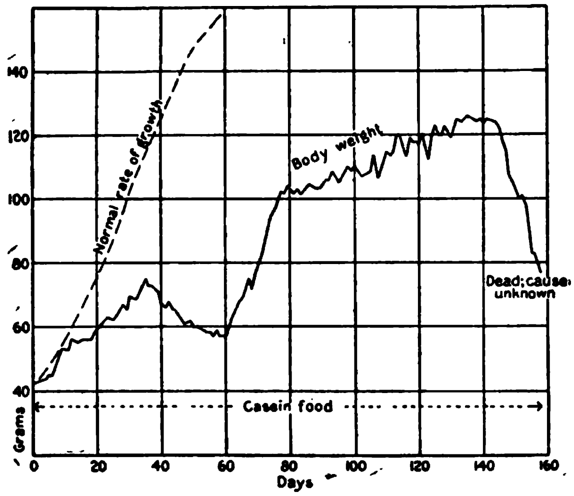


Chart XXXVIII (rat 50) shows maintenance for 158 days on a diet in which casein formed the sole protein. The composition of the food was as shown herewith:

	p. ct.
Casein........	18.0
Starch.......	29.5
Sugar........	15.0
Agar.........	5.0
Salt mixture I.	2.5
Lard.........	30.0

CHART XXXIX.

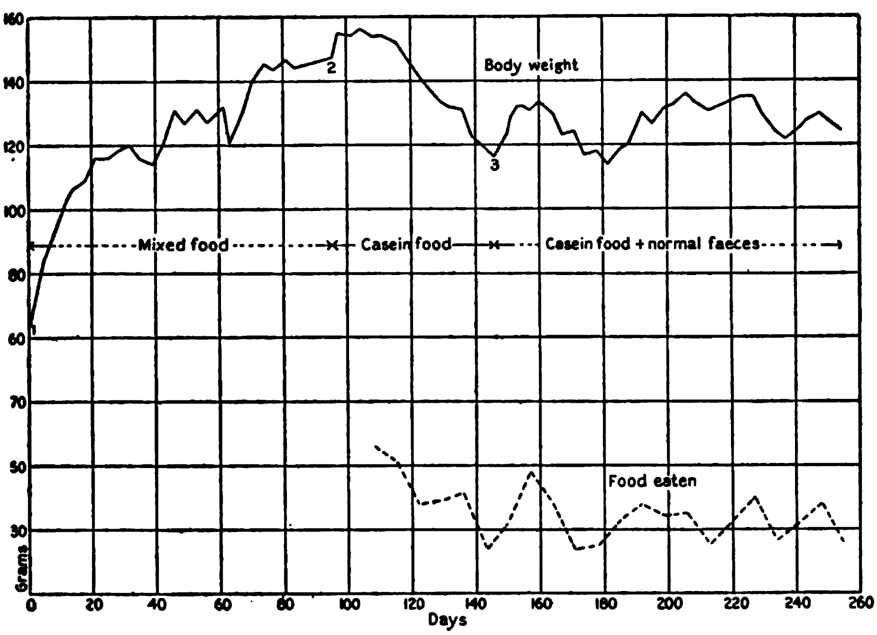


Chart XXXIX (rat 145, female) shows the effect of feeding a diet of isolated food substances in which casein formed the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 3. As shown by the food intake, the favorable effect is not due to an increased consumption of food. The diet during period 1 consisted of mixed food; during periods 2 and 3 as shown in table.

Periods 2 and 3.

	p. ct.	
Casein..........	18.0	....
Starch..........	32.5	....
Sugar...........	21.9 to 26.9	
Agar............	10.0	5.0
Salt mixture I....	2.5	2.6
Lard...........	20.0	25.0

CHART XL.

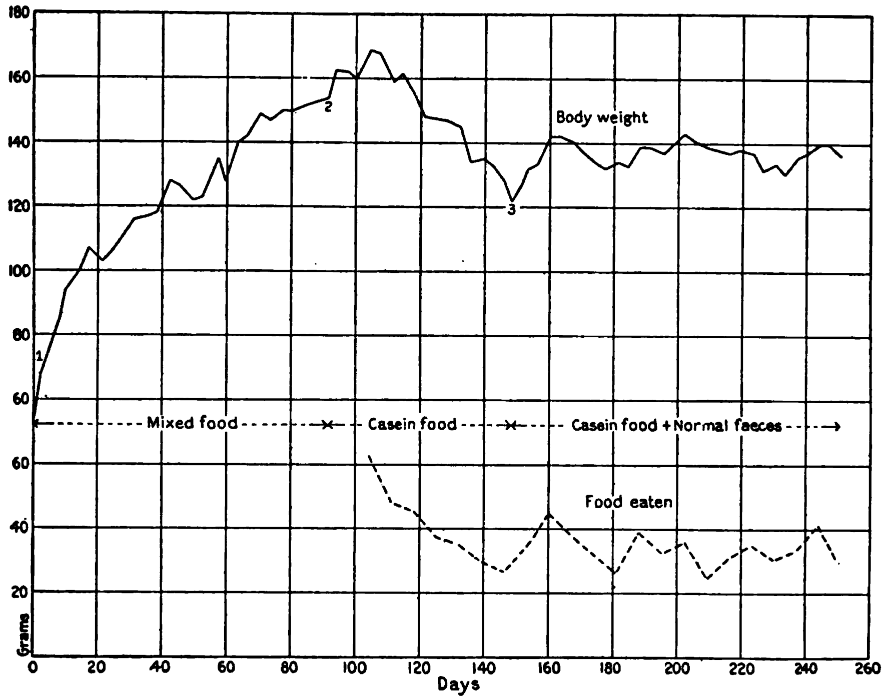


Chart XL (rat 150, female) shows the influence of a diet containing a mixture of isolated foodstuffs in which casein was the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 3. As shown by the food intake the favorable effect is not due to an increased consumption of food. The diet during period 1 consisted of mixed food; during periods 2 and 3 as shown herewith.

Periods 2 and 3.

	p. ct.	
Casein..........	18.0	....
Starch..........	32.5	....
Sugar..........	21.9 to 26.9	
Agar..........	0.0	5.0
Salt mixture I....	2.5	2.6
Lard..........	20.0	25.0

Chart XLI (rat 127, male) shows the influence of a diet containing a mixture of isolated foodstuffs in which casein was the sole protein. Period 1 represents the normal growth of the animal on a mixed food. The casein feeding began with period 2. The influence of fæces of normally fed animals in preventing for a time the decline in body-weight is shown during period 3. Period 4 shows the favorable nutritive influence of the substitution of protein-free milk for a part of the non-protein constituents of the diet. The diet during period 1 consisted of mixed food. During periods 2, 3, and 4 the composition of the food was as shown herewith.

Constituents.	Periods 2 and 3.		Constituents.	Per. 4.
	p. ct.			*p. ct.*
Casein..........	18.0	.....	Casein..........	18.0
Starch..........	32.5	.....	Protein-free milk....	28.2
Sugar..........	21.9 to 26.9		Starch..........	23.8
Salt mixture I....	2.6	.....	Agar..........	5.0
Lard..........	20.0	25.0	Lard..........	25.0

CHART XLI.

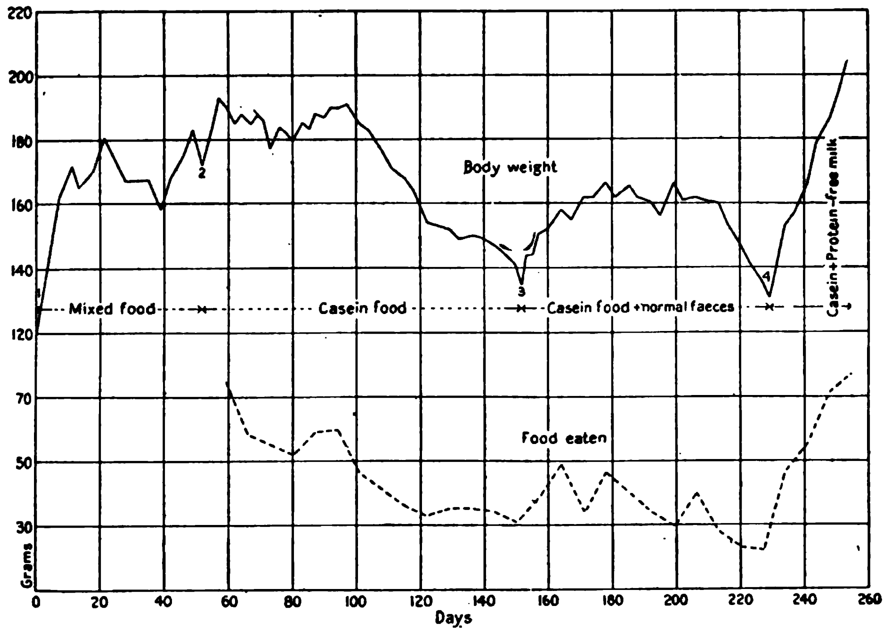


CHART XLII.

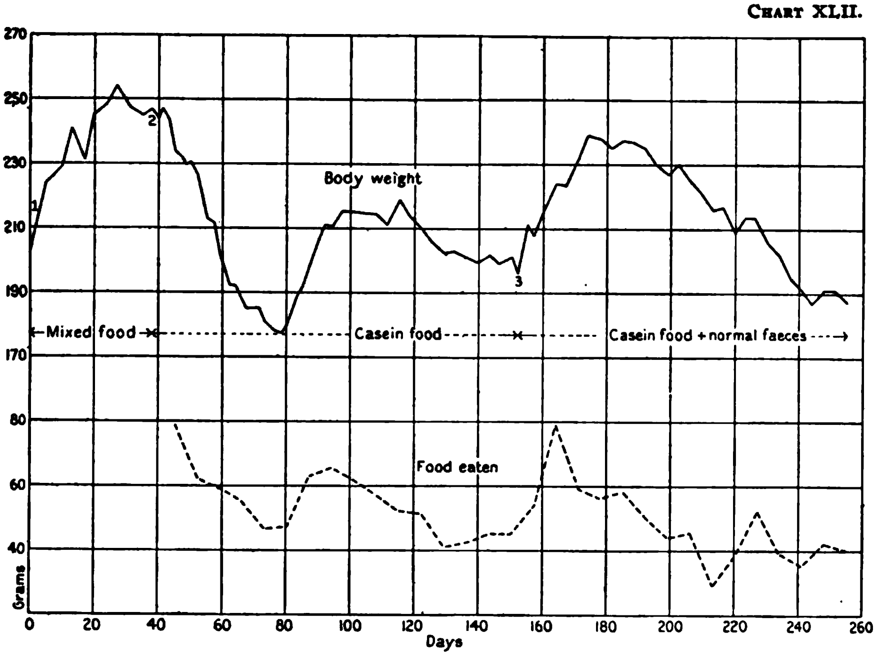


Chart XLII (rat 103, male) shows the influence of a diet of isolated food-stuffs containing casein as the sole protein. The satisfactory previous nutritive condition of the animal is shown during period 1 on mixed food. Casein feeding was begun with period 2; and the favorable effect of fæces of normally fed animals is shown during period 3. The composition of the food in periods 2 and 3 was as shown in table.

	p. ct.	
Casein	18.00	......
Starch	25.00 to	32.50
Sugar	12.87	25.37
Agar	0.00	5.00
Salt mixture	4.13	......
Lard	20.00	35.00

The salt mixture, which was prepared for other purposes, consisted of the citrates of calcium, magnesium, sodium, potassium, and iron, and the chlorides of sodium and potassium.

CHART XLIII.

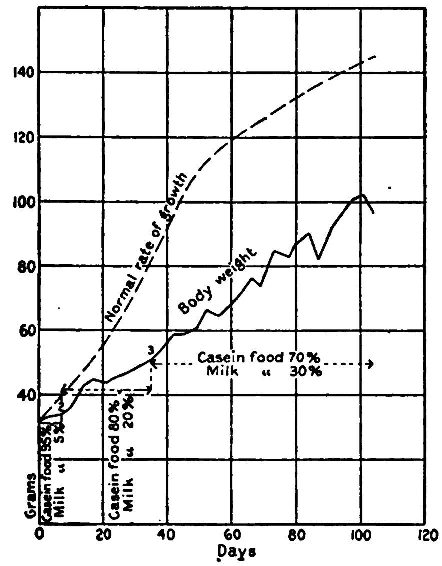


CHART XLIV.

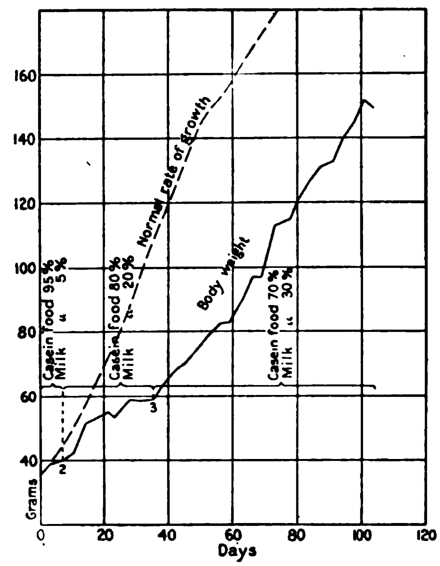
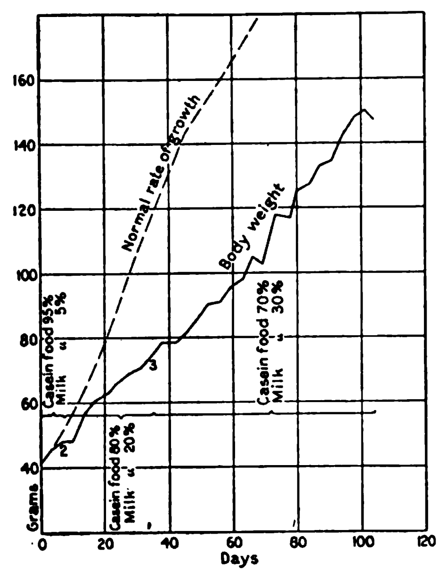


CHART XLV.



Charts XLIII (rat 231, female), XLIV (rat 230, male), and XLV (rat 223, male) show the effect of successive additions of increasing quantities of milk powder to the usual casein diet. The smaller quantities of milk are insufficient to induce normal growth. The diet during the several periods was as follows:

Constituents.	Per. 1.	Per. 2.	Per. 3.
	p. ct.	p. ct.	p. ct.
*Casein food......	95	80	70
†Milk food.......	5	20	30

*Casein food: casein, 18.0; starch, 32.5; sugar 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.

†Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3.

CHART XLVI.

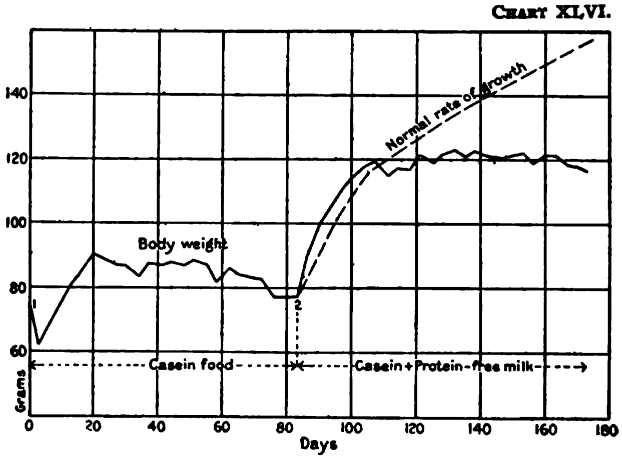
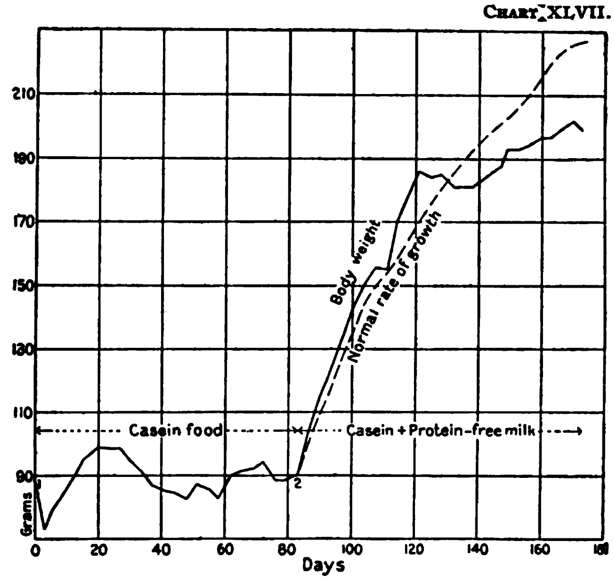


CHART XLVII.



Charts XLVI (rat 177, female) and XLVII (rat 191, male) show maintenance on a diet in which casein formed the sole protein during 83 days followed by growth when protein-free milk was substituted for a part of the non-protein constituents of the diet. The diet was as shown herewith.

Constituents.	Per. 1.	Per. 2.
	p. ct.	*p. ct.*
Casein	18.0	18.0
Protein-free milk	0.0	28.2
Starch	32.5	23.8
Sugar	17.0 to 20.0	0.0
Agar	5.0	5.0
Salt mixture I	2.5	0.0
Lard	22.0 25.0	25.0

CHART XLVIII.

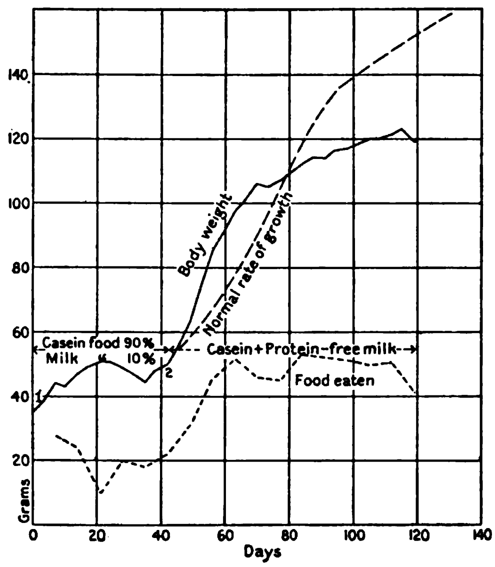
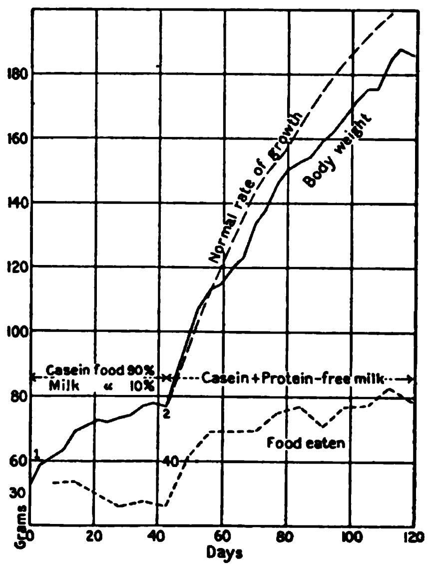


CHART XLIX.



Charts XLVIII (rat 210, female), XLIX (rat 209, male), L (rat 215, male), and LI (rat 216, male) show inadequate growth, during period 1, on the casein food with a small admixture of milk, followed by resumption of growth on a diet containing casein and protein-free milk in a quantity equivalent to that of our milk-paste diet which has proved sufficient to promote normal growth. The composition of the food was as shown in table.

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein food (casein, 18.0; starch, 32.5; sugar, 17.0; agar 5.0; salt mixture I, 2.5; lard, 25.0)..........	90	Casein.......	18.0
		Protein-free milk......	28.2
		Starch......	23.8
		Agar........	5.0
		Lard........	25.0
Milk food, (Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3).........	10		

CHART L.

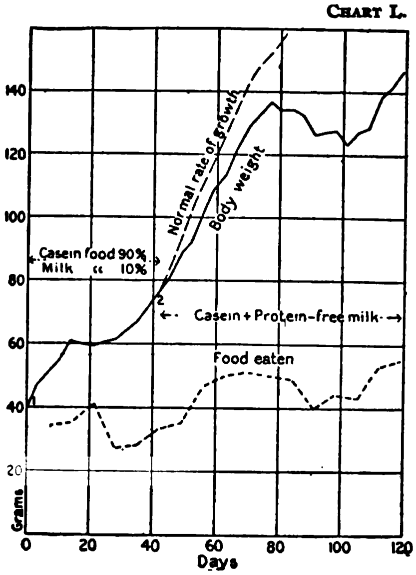


CHART LI.

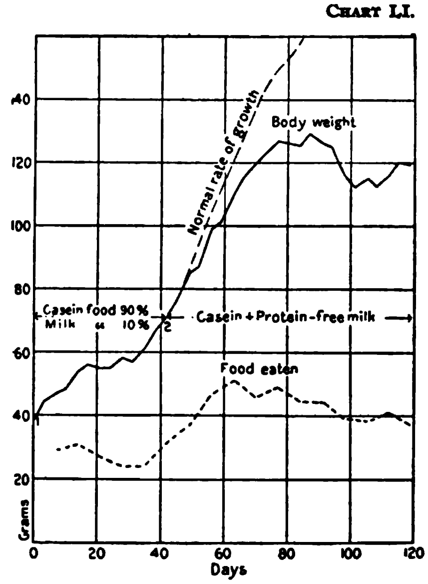


CHART LII.

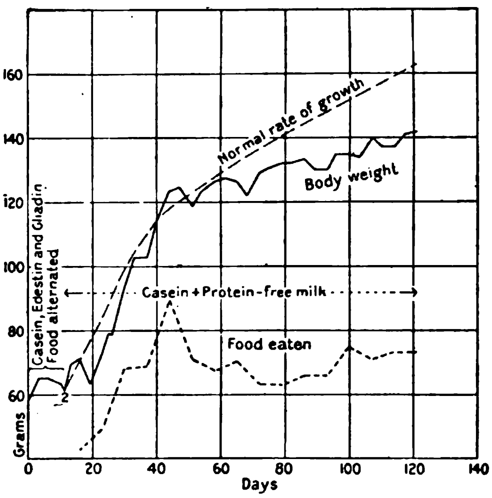
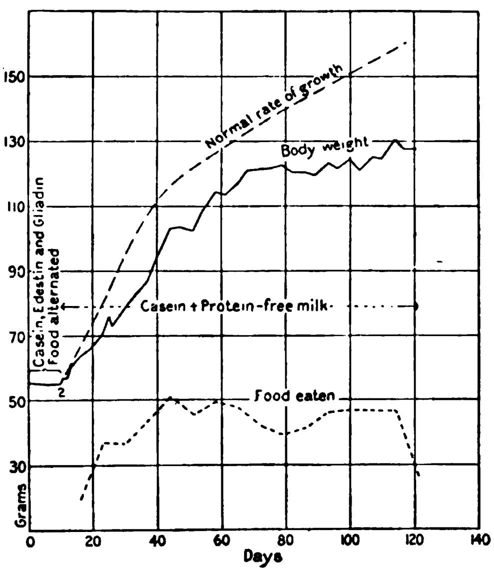


CHART LIII.



Charts LII (rat 205, female) and LIII (rat 207, female) show initiation of favorable growth when protein-free milk is added to a dietary containing casein as its sole protein in period 2. In the preliminary period an unsuccessful attempt was made to induce growth by feeding different proteins in rotation. The diet was as shown in table.

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein or Edestin or Gliadin	18.0	Casein	18.0
Starch	32.5	Protein-free milk	28.2
Sugar	17.0	Starch	23.8
Agar	5.0	Agar	5.0
Salt mixture I	2.5	Lard	25.0
Lard	25.0		

CHART LIV.

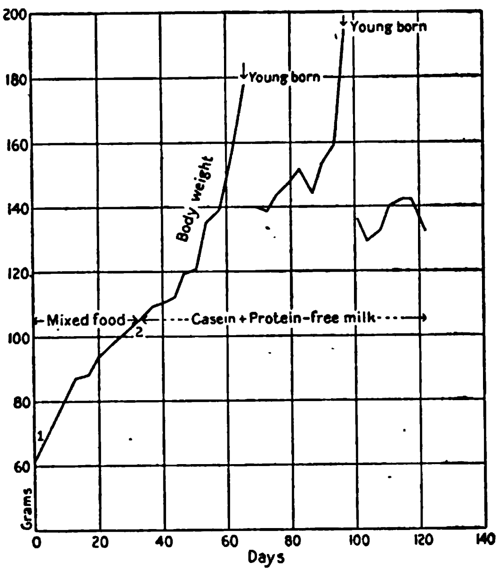


CHART LV.

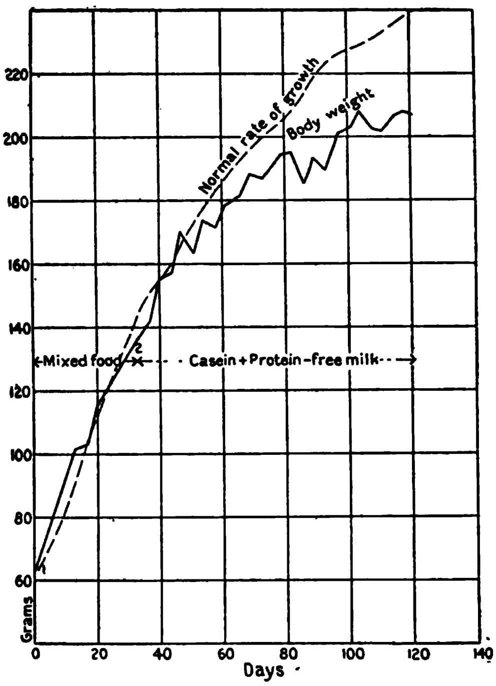


Chart LIV (rat 204, female) shows uninterrupted growth when a diet of isolated food-stuffs containing casein as its sole protein was substituted for mixed food. The requisite inorganic salts were furnished in the added protein-free milk. The experiment is of exceptional interest inasmuch as the animal successfully passed through two periods of pregnancy on a purine-free food containing a single protein. This obviously affords a method of studying various synthetic processes in the animal body. The diet during period 1 consisted of mixed food. During period 2 as shown herewith.

Period 2.	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

Chart LV (rat 203, male) shows uninterrupted growth when a diet of isolated foodstuffs containing casein as its sole protein was substituted for mixed food. The requisite inorganic salts were furnished in the added protein-free milk. The diet during period 1 consisted of mixed food; during period 2, as shown herewith.

Period 2.	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LVI.

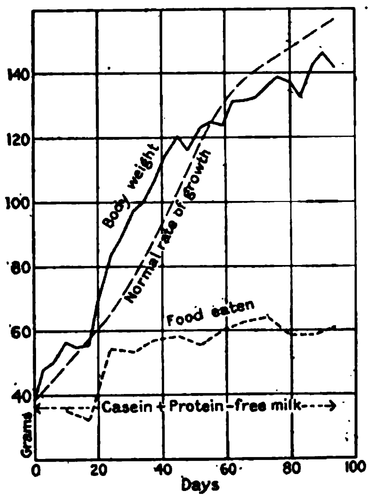


CHART LVII.

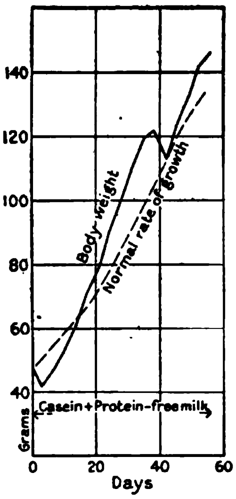


CHART LVIII.

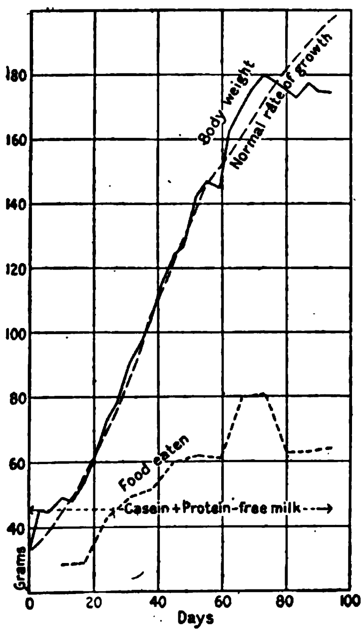


CHART LIX.

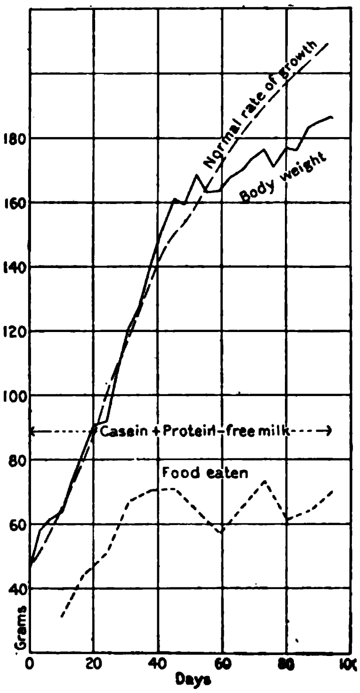
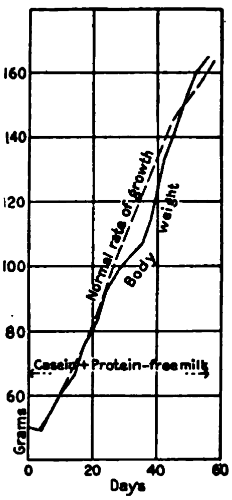


CHART LX.



Charts LVI (rat 238, female), LVII (rat 269, female), LVIII (rat 247, male), LIX (rat 252, male), and LX (rat 268, male) show normal growth on a diet containing a single protein, casein. The requisite inorganic salts were furnished in the added protein-free milk. This experiment illustrates artificial nutrition with isolated food-substances from a very early period of life. The diet was as shown herewith.

	p. ct.
Casein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXI.

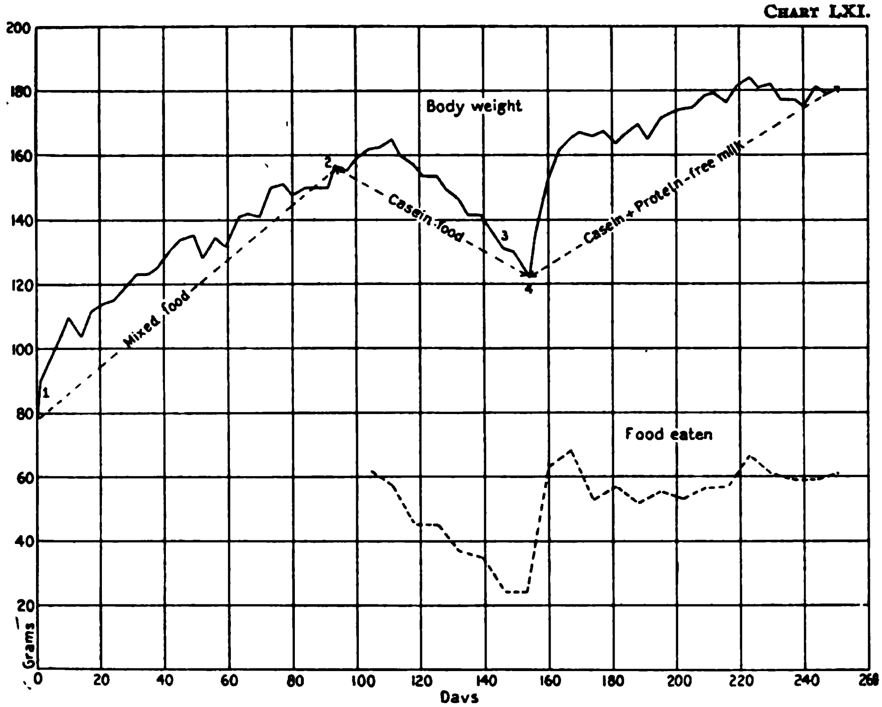
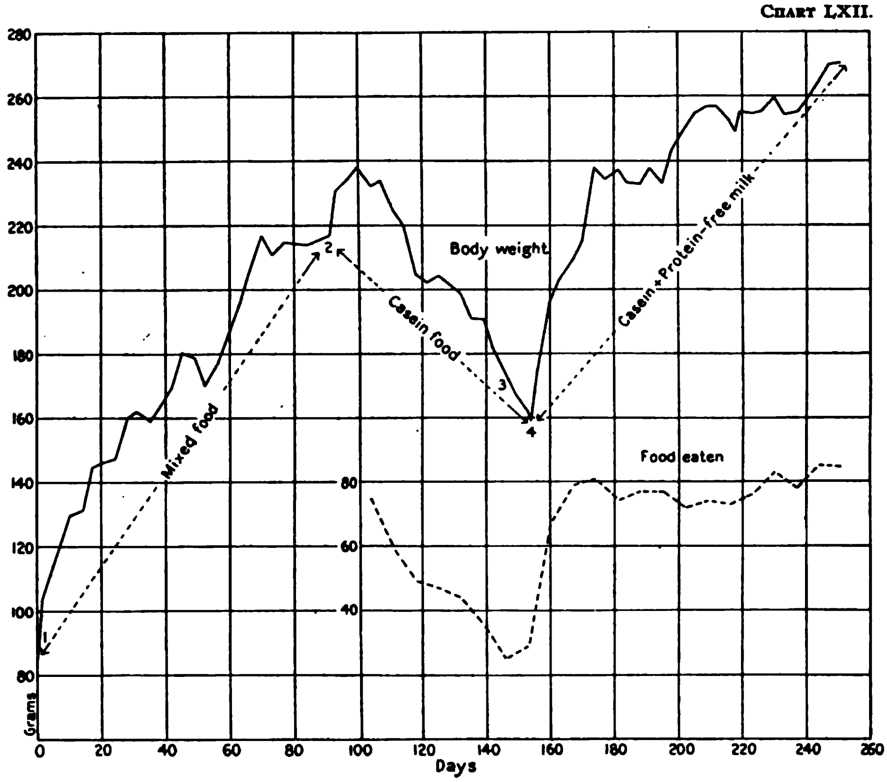


CHART LXII.



Charts LXI (rat 141, female) and LXII (rat 139, male) show recovery of animals maintained on a diet containing casein as the sole protein. The preliminary nutritive condition of the rats is shown to be satisfactory in period 1 on a mixed diet. The ultimate decline on the casein diet during period 2 could not be checked by increasing the content of casein during period 3. This shows that the nutritive failure of the animals was not attributable to the protein *per se*. Speedy recuperation and maintenance attended the substitution of protein-free milk for the inorganic salt mixture contained in food previously used. Note the influence of this dietary change on the appetite of the animals. In period 1 mixed food was used. The composition of food, during the other periods was as shown in table.

Constituents.	Per. 2.		Per. 3.	Per. 4.
	p. ct.		*p. ct.*	*p. ct.*
Casein	18.0	.....	36.0	18.0
Protein-free milk	0.0	.....	0.0	28.2
Starch	32.5	.....	22.5	23.8
Sugar	21.9 to	26.9	13.9	0.0
Agar	0.0	5.0	0.0	5.0
Salt mixture I	2.5	2.6	2.6	0.0
Lard	20.0	25.0	25.0	25.0

CHART LXIII.

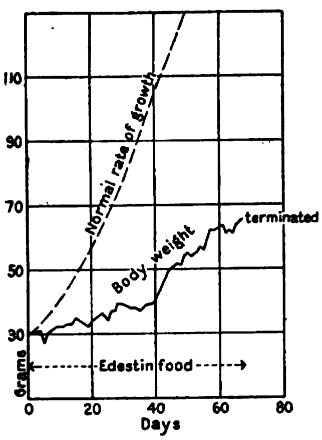
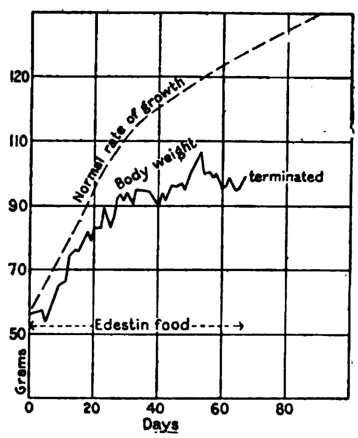


CHART LXIV.



	p. ct.
Edestin	18.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I.	2.5
Lard	30.0

Charts LXIII (rat 60, male) and LXIV (rat 58, female) show maintenance and slight growth of a rat on a diet in which edestin constituted the sole protein for 67 days. The experiment was terminated because of the death of another animal, which was found partly eaten, in the same cage. The diet was as shown herewith.

CHART LXV.

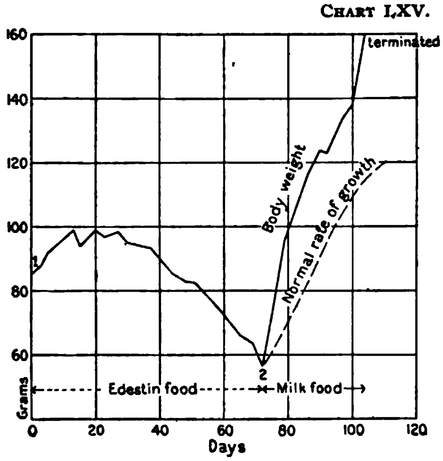
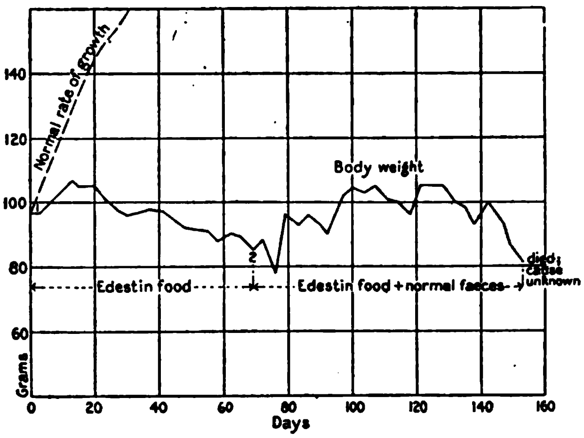


Chart LXV (rat 189, female) shows failure of rat to grow or be maintained on a diet containing edestin as the sole protein during 72 days (period 1). There is no loss of capacity to grow, as will be seen by the curve of growth on the milk diet in period 2, 32 days. The diet consisted of—

	p. ct.
Period 1.	
Edestin	18.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I	2.5
Lard	30.0
Period 2.	
Trumilk	60.0
Starch	15.7
Salt mixture I	1.0
Lard	23.3

CHART LXVI.



Charts LXVI (rat 169, male) and LXVII (rat 190, male) show maintenance on a diet in which edestin formed the sole protein. The influence of fæces of normally fed animals in preventing decline in body-weight for some time is shown during period 2. The fæces were obtained from rats temporarily introduced into the cage each day. The diet is given above.

	p. ct.	
Edestin	18.0	....
Starch	29.5 to	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture, I	2.5	....
Lard	25.0	30.0

CHART LXVII.

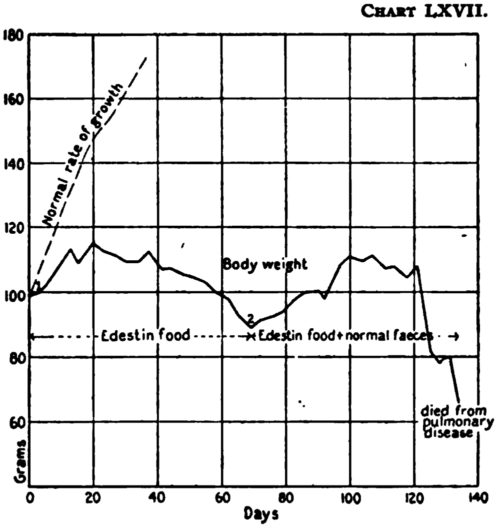
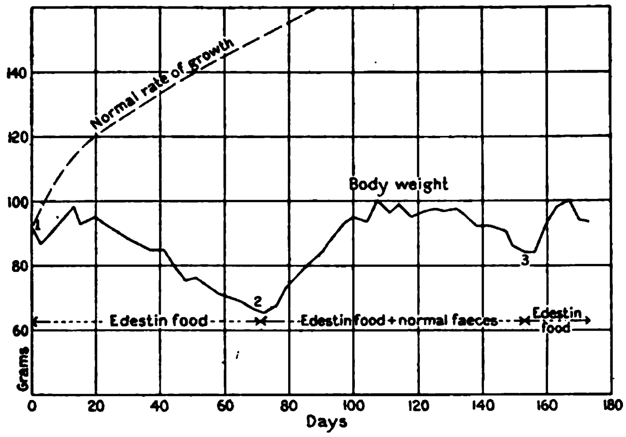


CHART LXVIII.



Charts LXVIII (rat 196, female) and LXIX (rat 193, female) show maintenance on a diet in which edestin formed the sole protein. The influence of fæces of normally fed animals in preventing decline in body-weight is shown during period 2. The giving of fæces was discontinued during period 3. The fæces were obtained from normally fed rats temporarily introduced into the cage each day. The diet is given above.

	p. ct.	
Edestin	18.0	....
Starch	29.5 to	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART LXIX.

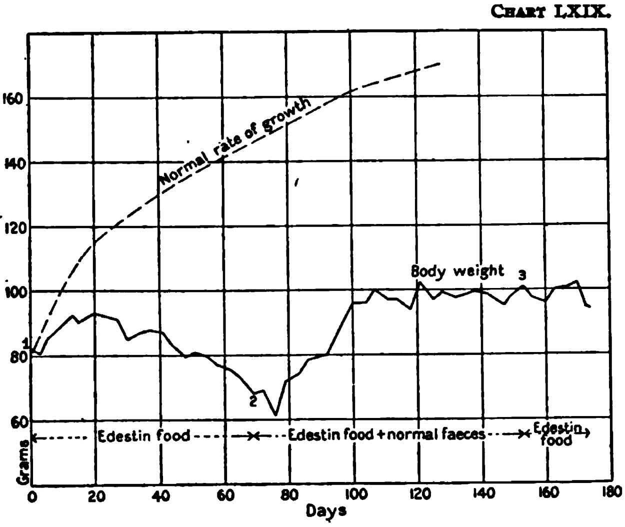


CHART LXX.

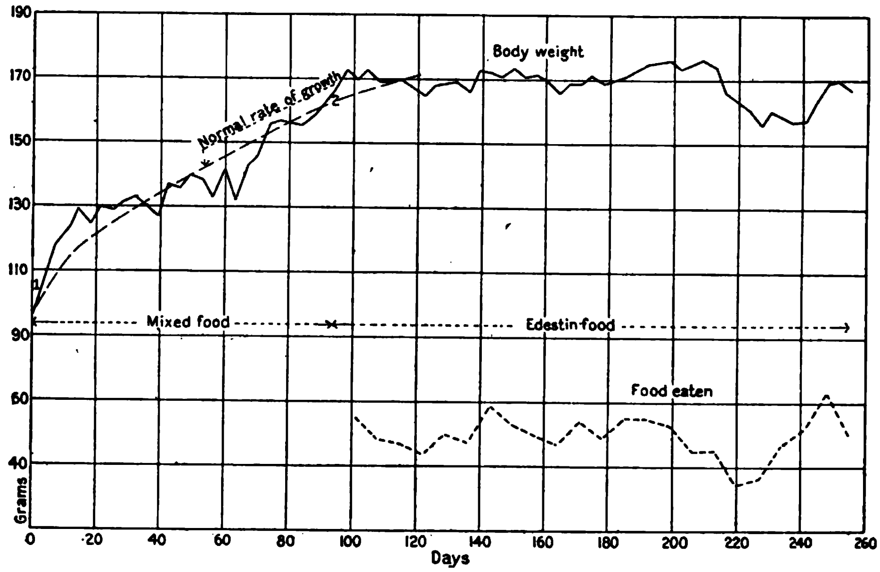


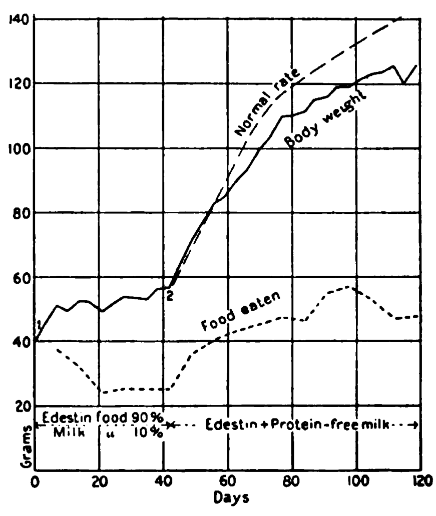
Chart LXX (rat 133, female) shows maintenance on a diet in which edestin was the sole protein during 161 days. Period 1 on a mixed diet shows normal growth. Period 2 is of interest because the food was also purine-free and devoid of organically combined phosphorus. All growth ceased during the edestin feeding (period 2), in contrast with other experiences where protein-free milk was present in the dietary.

Period 2.

	p. ct.	
Edestin	18.0	....
Starch	29.5 to	32.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

Chart LXXI (rat 218, female) shows inadequate growth on a diet of edestin+milk-paste (period 1) followed by growth during period 2, in which the food contained protein-free milk and edestin as its sole protein. In growing to several times its original weight the animal must have synthesized its purine- and phosphorus-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curve. The diet consisted of—

CHART LXXI.



Period 1.

	p. ct.
Edestin food (edestin, 18.0; starch, 32.5; sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0)	90
Milk food (Trumilk, 60.0; starch 15.7; salt mixture I, 1.0; lard, 23.3.)	10

Period 2.

Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXXII.

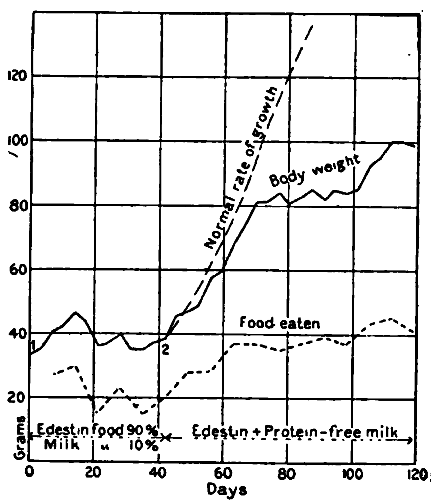


CHART LXXIV.

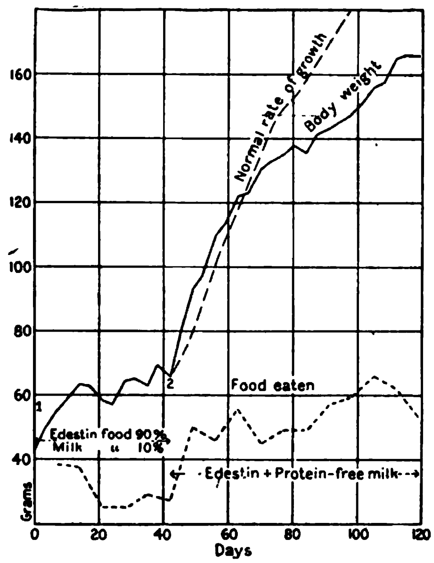
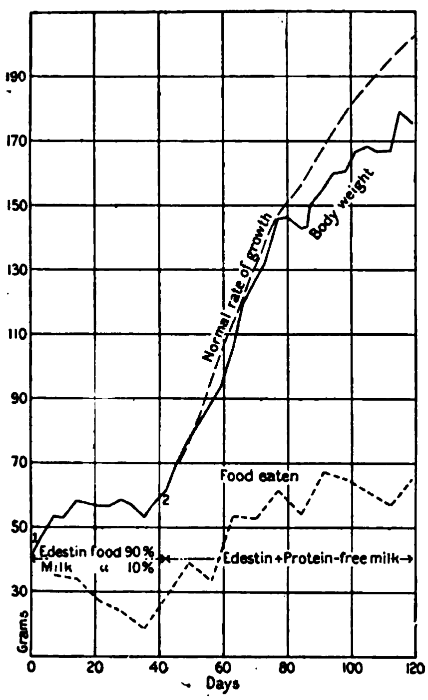


CHART LXXIII.



Charts LXXII (rat 217, male), LXXIII (rat 211, male), and LXXIV (rat 212, male) show inadequate growth on a diet of edestin + milk-paste (period 1) followed by growth during period 2, in which the food contained protein-free milk and edestin as its sole protein. It should be noted that the animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food intake curve. The diet consisted of—

Period 1.	p. ct.
Edestin food (edestin, 18.0; starch, 32.5; sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0)	90.0
Milk food (Trumilk, 60.0; starch; 15.7, salt mixture I, 1.0; lard,, 23.3)	10.0
Period 2.	
Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART LXXV.

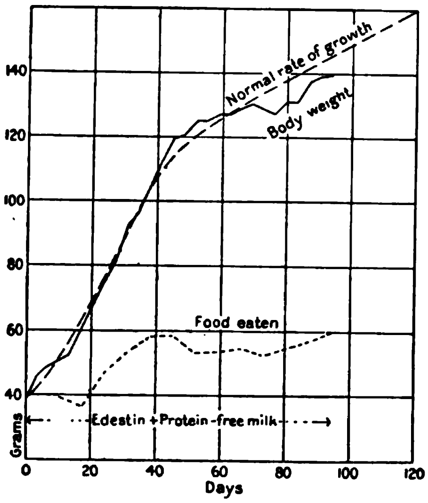
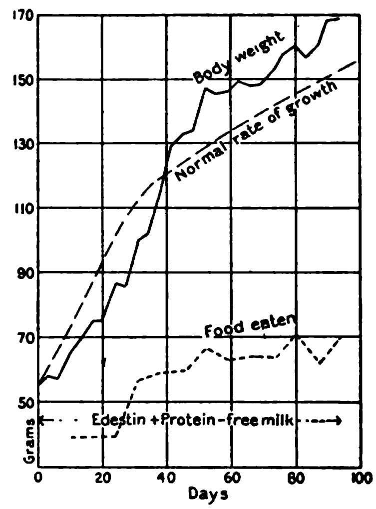


CHART LXXVI.



Charts LXXV (rat 248, female) and LXXVI (rat 253, female) show growth from an early age on a diet containing protein-free milk in which edestin formed the sole protein. It should be noted that the animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curve. The diet was as shown herewith.

	p. ct.
Edestin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

Chart LXXVII (rat 114, male) shows the failure of edestin (period 2) to maintain previous satisfactory nutritive condition of the animal during period 1, on mixed food, even after adding fæces to the diet (period 3). Immediate improvement and satisfactory nutritive condition followed addition of protein-free milk to edestin food (period 4). The diet consisted of mixed food for period 1, and for periods 2, 3, and 4 was as shown in table.

Constituents.	Periods 2 and 3.		Constituents.	Per. 4.
	p. ct.			p. ct.
Edestin	18.0	.....	Edestin	18.0
Starch	29.5 to 32.5		Protein-free milk	28.2
Sugar	15.0	17.0	Starch	23.8
Agar	5.0	.....	Agar	5.0
Salt mixture I	2.5	.....	Lard	25.0
Lard	25.0	30.0		

Chart LXXVIII (rat 140, female) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. The diet consisted of mixed food for period 1, and for periods 2 and 3 it was as shown in table.

Constituents.	Per. 2.		Per. 3.
	p. ct.		p. ct.
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to 32.5		23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXVII.

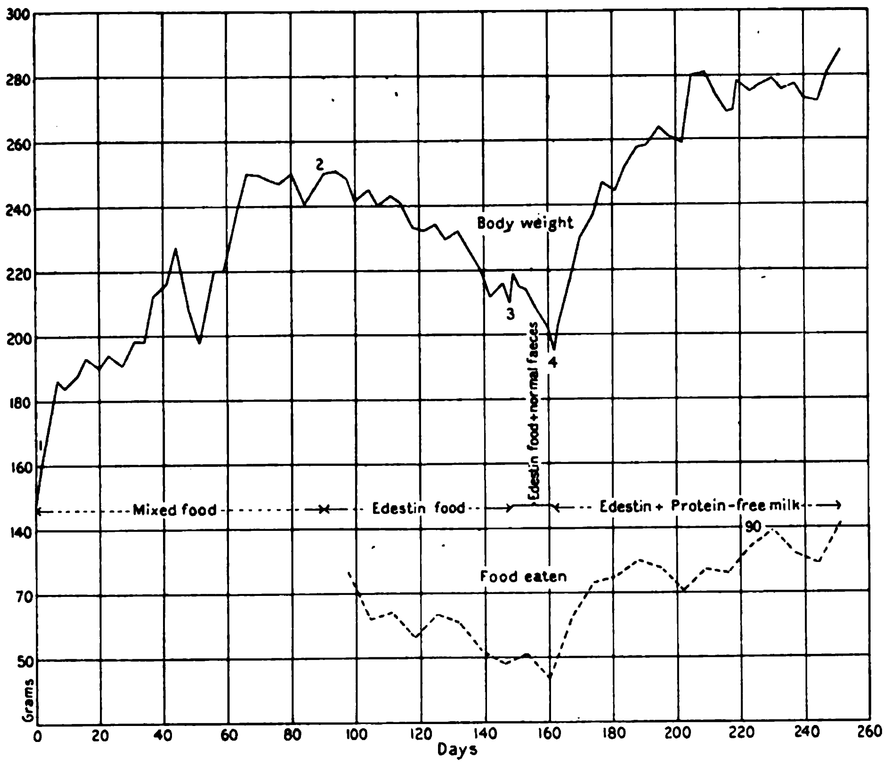


CHART LXXVIII.

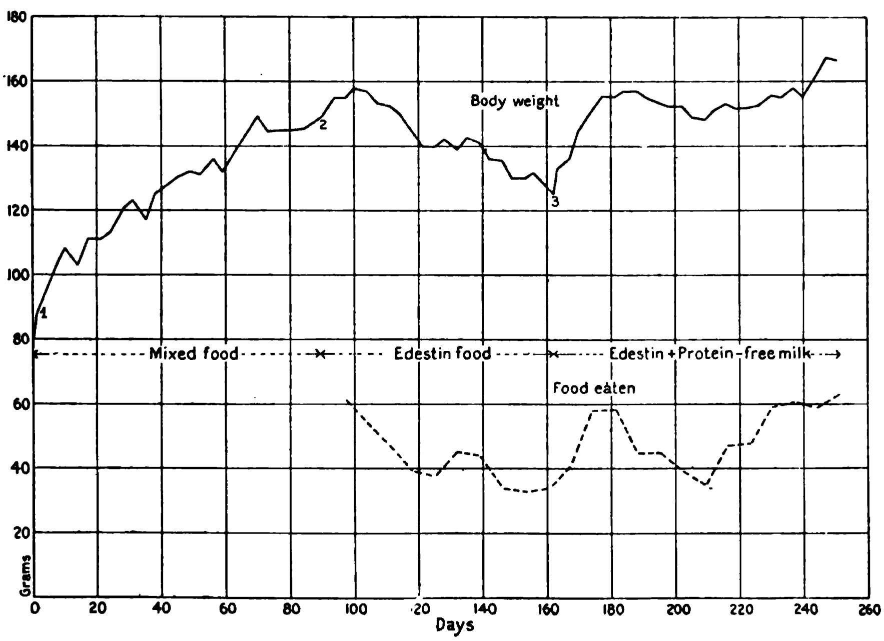


CHART LXXIX.

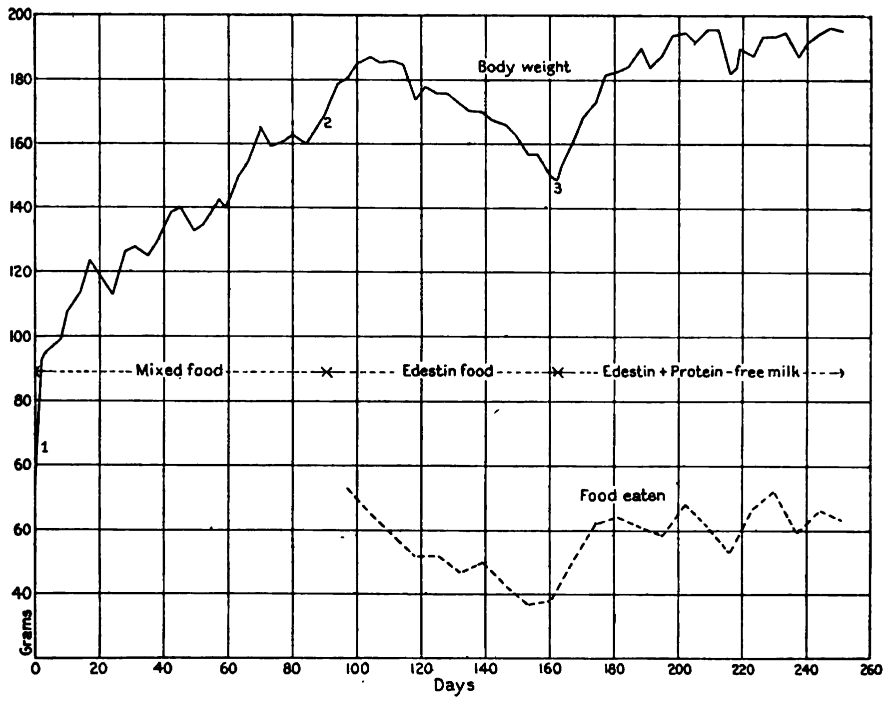


Chart LXXIX (rat 152, female) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. The diet consisted of mixed food for period 1 and for periods 2 and 3 was as follows:

Constituents.	Per. 2.		Per. 3.
	p. ct.		*p. ct.*
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to 32.5		23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXX.

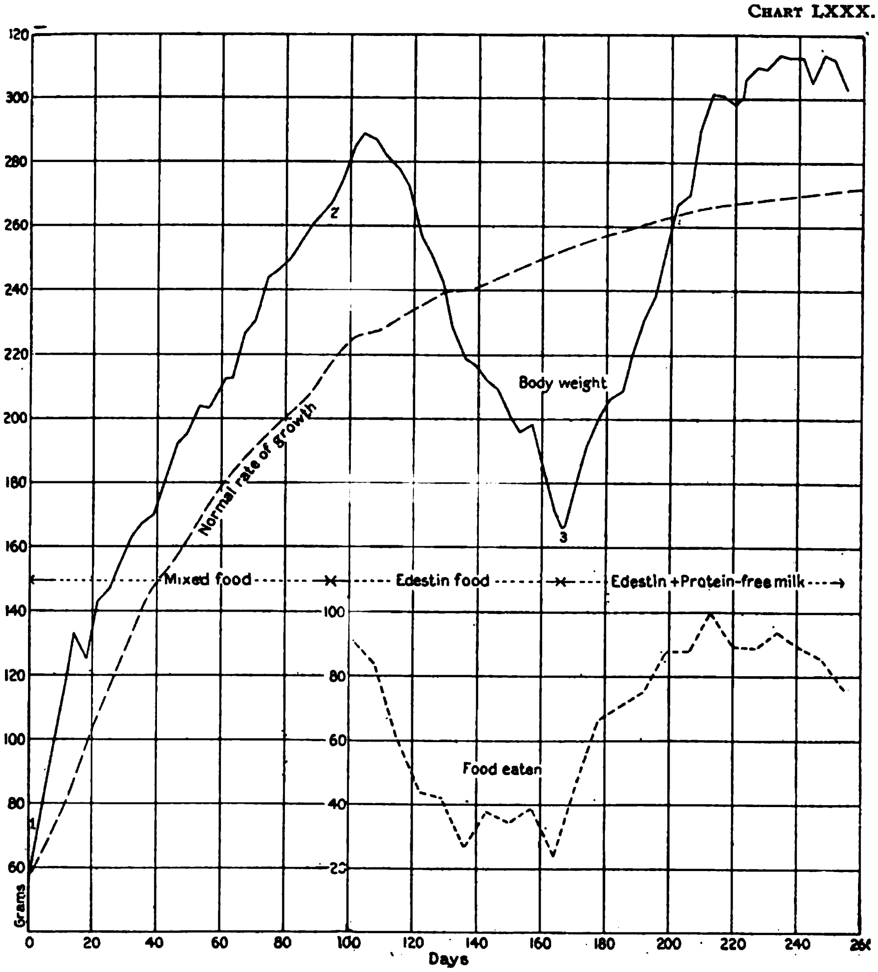


Chart LXXX (rat 148, male) shows the failure of maintenance on a diet in which edestin formed the sole protein (period 2), until protein-free milk was added to the diet (period 3). Period 1, on mixed food, is introduced to show the previous satisfactory nutritive condition of the animal. Note the influence of changes in diet on the food consumption. The diet consisted of mixed food for period 1, and for periods 2 and 3 was as follows:

Constituents.	Per. 2.		Per. 3.
	p. ct.		*p. ct.*
Edestin	18.0	.....	18.0
Protein-free milk	0.0	.....	28.2
Starch	29.5 to 32.5		23.8
Sugar	15.0	17.0	0.0
Agar	5.0	.....	5.0
Salt mixture I	2.5	.....	0.0
Lard	25.0	30.0	25.0

CHART LXXXI.

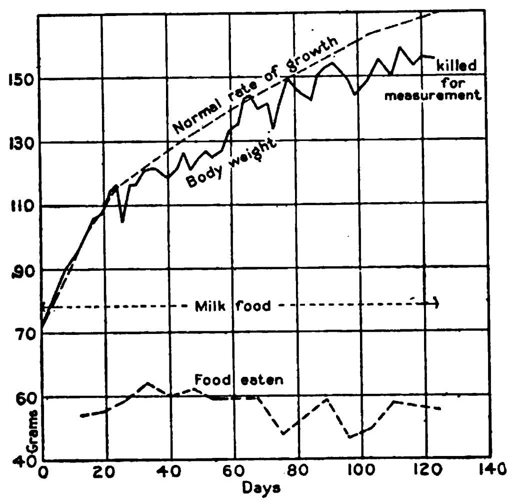
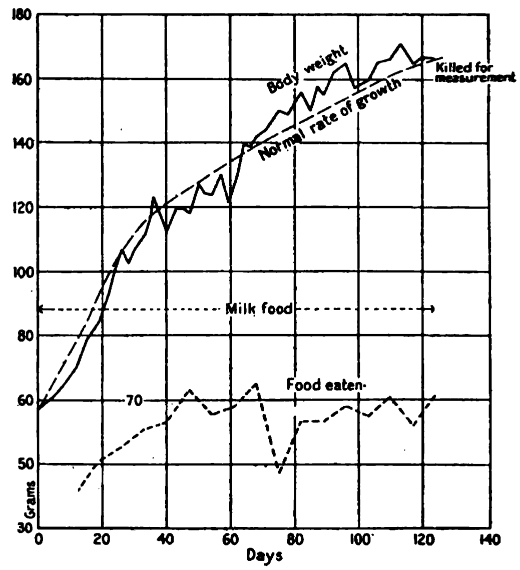


CHART LXXXII.



Charts LXXXI (rat 96, female) and LXXXII (rat 97, female). Control animals for the glutenin dwarfs, Charts LXXXIV–LXXXVI. For other data see page 73. The diet consisted of Trumilk, 60 p. ct.; starch, 15.7 p. ct.; salt mixture I, 1 p. ct., lard, 23.3 p. ct.

CHART LXXXIII.

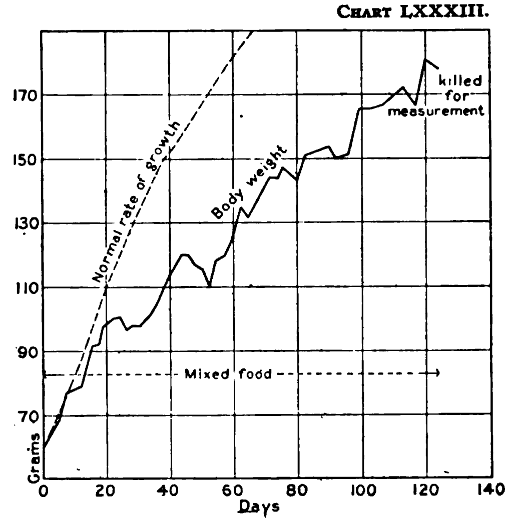


Chart LXXXIII (rat 99, male). Control animal for the glutenin dwarfs, Charts LXXXIV–LXXXVI. For other data see page 73. The diet consisted of mixed food.

Chart LXXXIV.

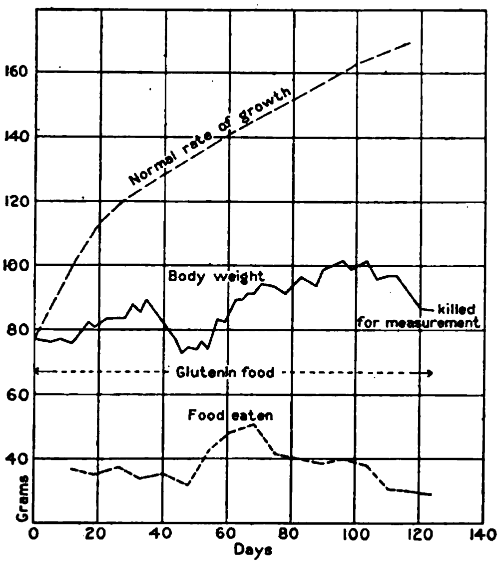


Chart LXXXV.

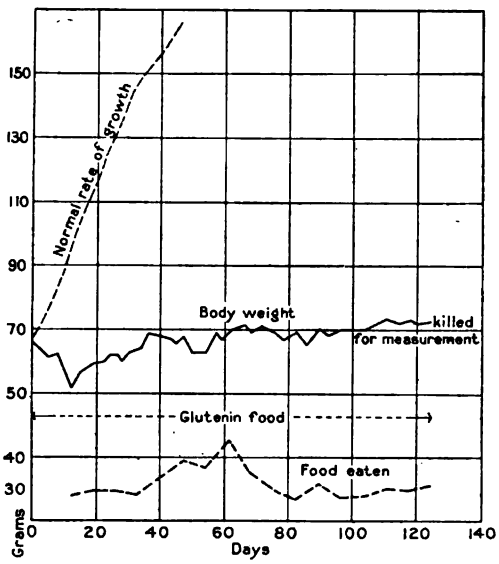
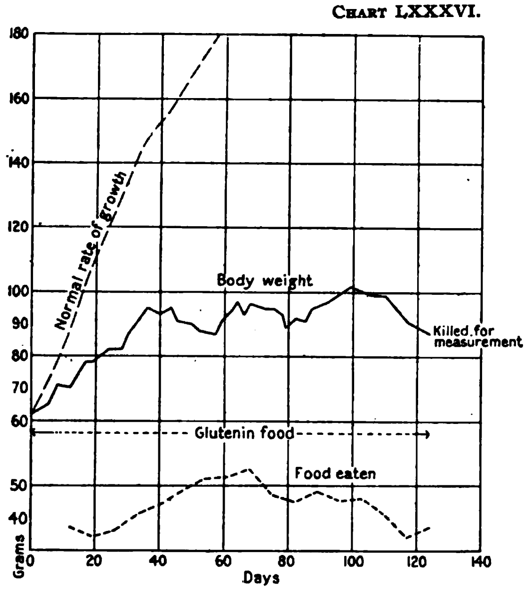


Chart LXXXVI.



	p. ct.	
Glutenin........	18.0	....
Starch..........	14.5 to	34.5
Sugar...........	15.0	20.0
Agar............	5.0	....
Salt mixture I....	2.5	....
Lard...........	20.0	45.0

Charts LXXXIV (rat 100, female), LXXXV (rat 101, male), and LXXXVI (rat 102, male). These animals, from the same family as the control rats, Charts LXXXI–LXXXIII, were maintained on a diet of glutenin from wheat 124 days, when they were killed for measurement. The chart illustrates maintenance without appreciable growth. For other data see page 73. The diet was as shown herewith.

CHART LXXXVII.

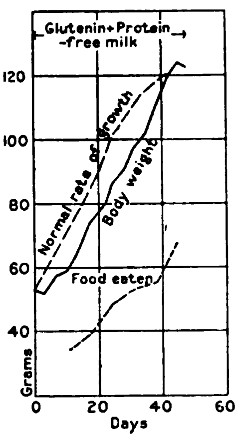


CHART LXXXVIII.

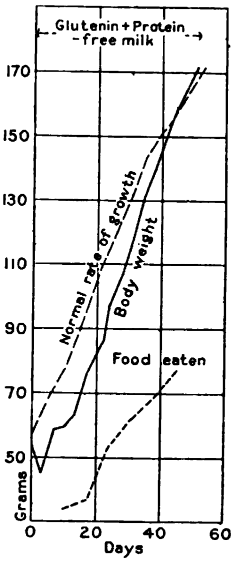
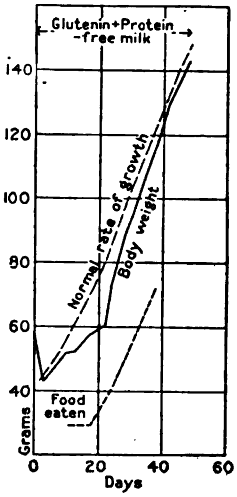


CHART LXXXIX.



Charts LXXXVII (rat 293, female), LXXXVIII (rat 284, male), and LXXXIX (rat 279, male) show growth from an early age on a diet containing protein-free milk, in which glutenin from wheat formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curves. The diet was as shown in table.

	p. ct.
Glutenin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XC.

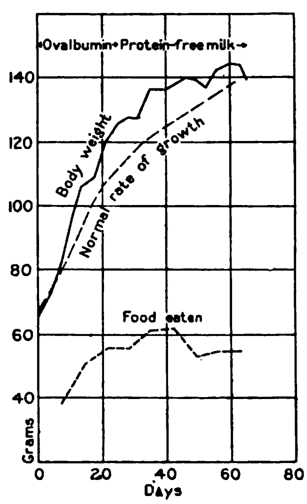
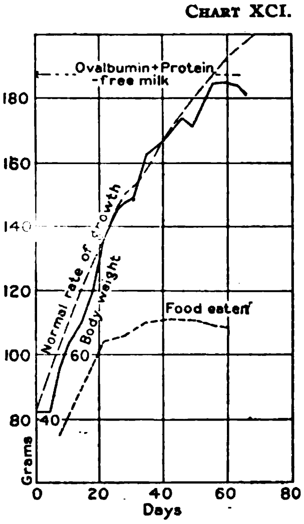


CHART XCI.



Charts XC (rat 258, female) and XCI (rat 250, male) show growth from an early age on a diet containing protein-free milk, in which ovalbumin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curves. The diet was as shown above.

	p. ct.
Ovalbumin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XCII.

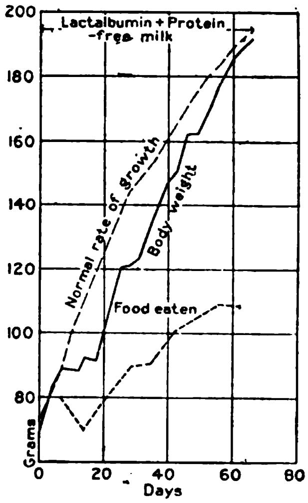
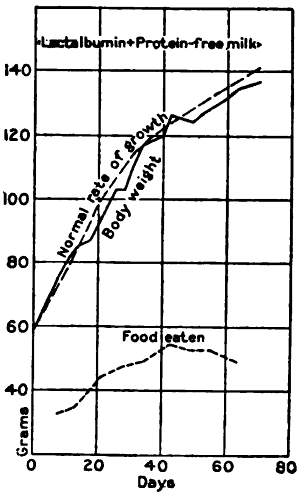


CHART XCIII.



Charts XCII (rat 251, male) and XCIII (rat 259, female) show growth from an early age on a diet containing protein-free milk, in which lactalbumin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine- and phosphorus-containing complexes from purine-free food. The influence of size on the food requirement is shown by the food-intake curves. The diet was as shown herewith.

	p. ct.	
Lactalbumin	18.0	....
Protein-free milk	28.2	....
Starch	16.8 to 18.8	
Agar	5.0	....
Lard	30.0	32.0

CHART XCIV.

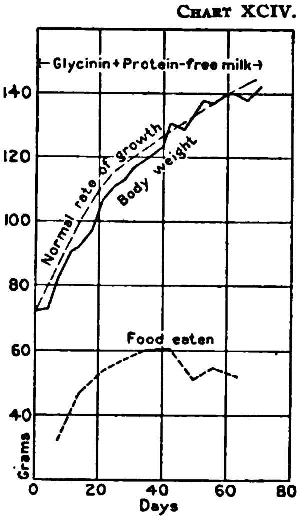
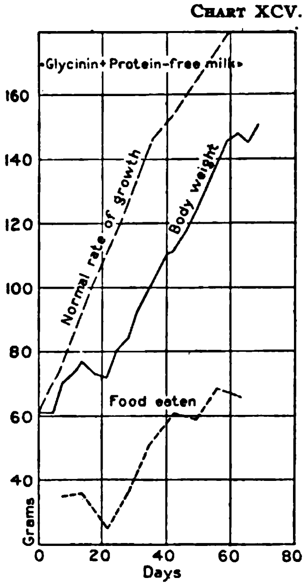


CHART XCV.



Charts XCIV (rat 257, female) and XCV (rat 241, male) show growth from an early age on a diet containing protein-free milk, in which glycinin formed the sole protein. The animals in growing to several times their original weight must have synthesized their purine-containing complexes from purine-free food. The influence of size on food requirement is shown by the food-intake curves. The diet was as shown herewith.

	p. ct.
Glycinin	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART XCVI.

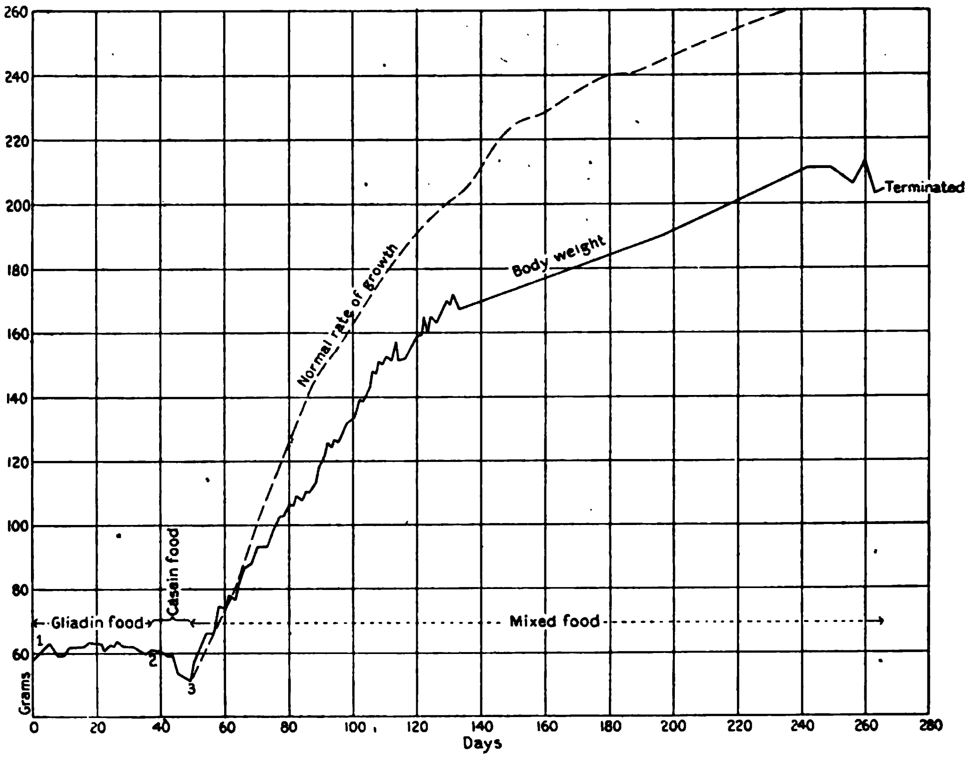


Chart XCVI (rat 36, male) shows the failure of inhibition of growth to check the "capacity to grow." The rat was stunted on gliadin food for 37 days (period 1) and on casein food for 12 days (period 2) and completely recovered growth on mixed diet during 217 days (period 3). The diet for periods 1 and 2 was as follows:

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		p. ct.
Gliadin (from wheat)	18.0	Casein	18.0
Starch	29.5	Starch	29.5
Sugar	15.0	Sugar	15.0
Agar	5.0	Agar	5.0
Salt mixture I	2.5	Salt mixture I	2.5
Lard	30.0	Lard	30.0

Chart XCVII (rat 37, male) shows unimpaired capacity for growth on mixed diet and milk diet after an earlier period of stunted growth on gliadin diet for 37 days (period 1) and casein diet for 12 days (period 2). Part of the period of growth was accomplished on milk food, part on mixed food, the change being made at 3 to mixed food, at 4 to milk food, and at 5 to

mixed food again. Note that this has not affected the typical character of the curve of growth. The diet was as follows:

Constituents.	Per. 1.	Constituents.	Per. 2.	Periods 3 and 5.	Constituents.	Per. 4.
	p. ct.		p. ct.			p. ct.
Gliadin (from wheat)	18.0	Casein	18.0	Mixed food.	Trumilk	60.0
Starch	29.5	Starch	29.5		Starch	16.7
Sugar	15.0	Sugar	15.0		Lard	23.3
Agar	5.0	Agar	5.0			
Salt mixture I	2.5	Salt mixture I	2.5			
Lard	30.0	Lard	30.0			

CHART XCVII.

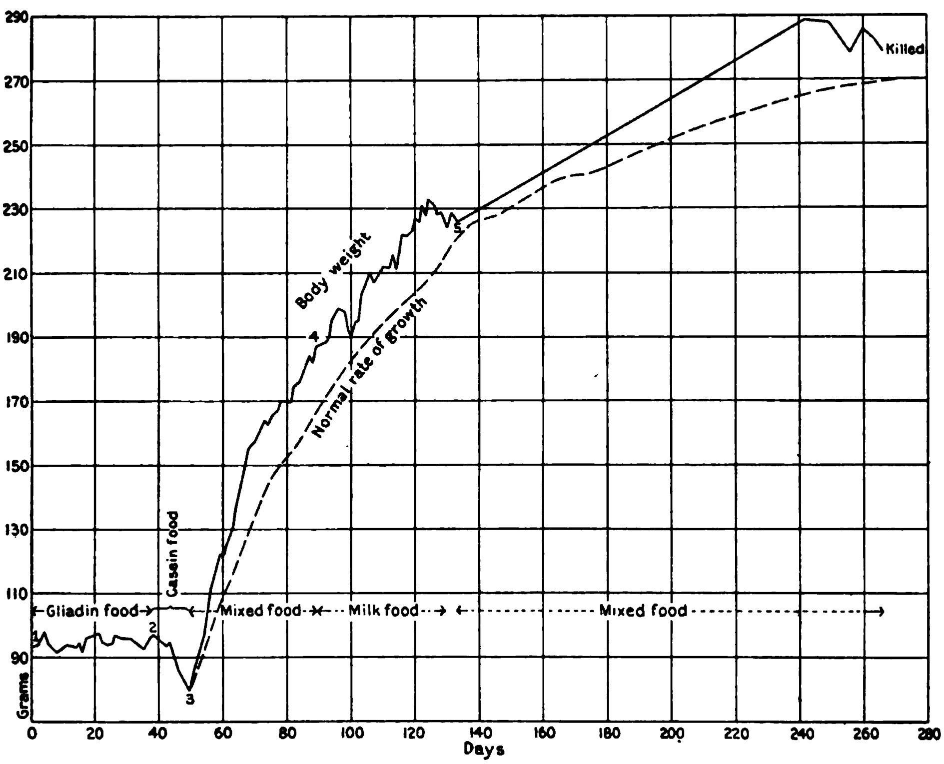


CHART XCVIII.

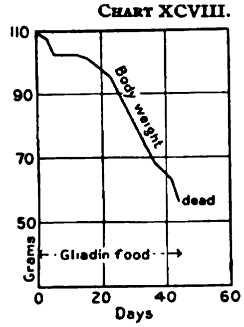


Chart XCVIII (rat 185, male) shows the failure of a rat to be maintained on a diet composed as shown herewith.

	p. ct.
Gliadin (from wheat)	18.0
Starch	29.5
Sugar	15.0
Agar	5.0
Salt mixture I	2.5
Lard	30.0

CHART XCIX.

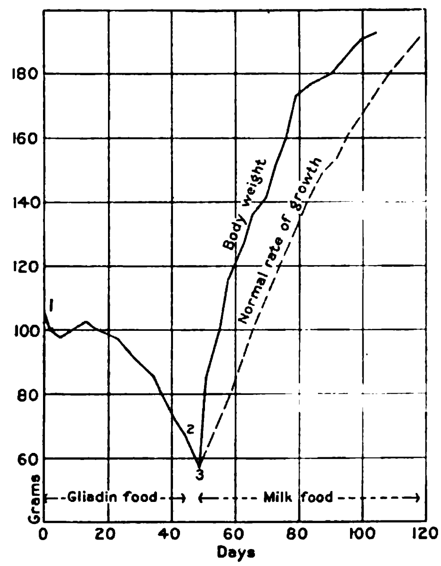
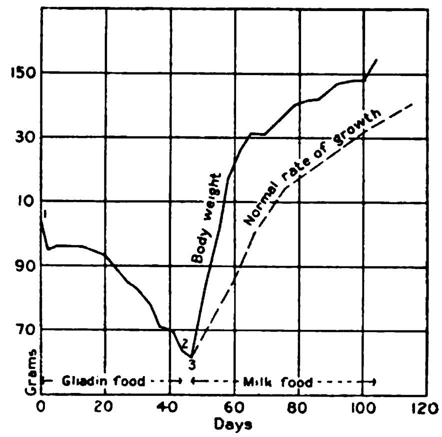


CHART C.



Charts XCIX (rat 186, male) and C (rat 188, female) show the failure of the rat to be maintained during periods 1 and 2 on diets mentioned below. The perfect resumption of growth when the diet consisted of milk-paste (period 3) illustrates that the "capacity to grow" normally is not visibly impaired by previous large loss of body-weight. The food consisted of—

Constituents.	Per. 1.	Per. 2.	Constituents.	Per. 3.
	p. ct.	*p. ct.*		*p. ct.*
Gliadin (from wheat)	18.0	0.0	Trumilk	60.0
Edestin	0.0	18.0	Starch	15.7
Starch	29.5	32.5	Salt mixture I	1.0
Sugar	15.0	17.0	Lard	23.3
Agar	5.0	5.0		
Salt mixture I	2.5	2.5		
Lard	30.0	25.0		

Chart CI (rat 147, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2), until fæces were added in period 3. The diet consisted of mixed food during period 1; for periods 2 and 3 it was as shown herewith.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

Chart CII (rat 142, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2). The addition of fæces to the diet in periods 3 and 4 checked the decline. During period 3 the fæces added were thoroughly sterilized and seemed to be less efficient than the unsterilized fæces in period 4, or in other similar experiments. The diet consisted of mixed food during period 1; for periods 2, 3, and 4 it was as shown herewith.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART CI.

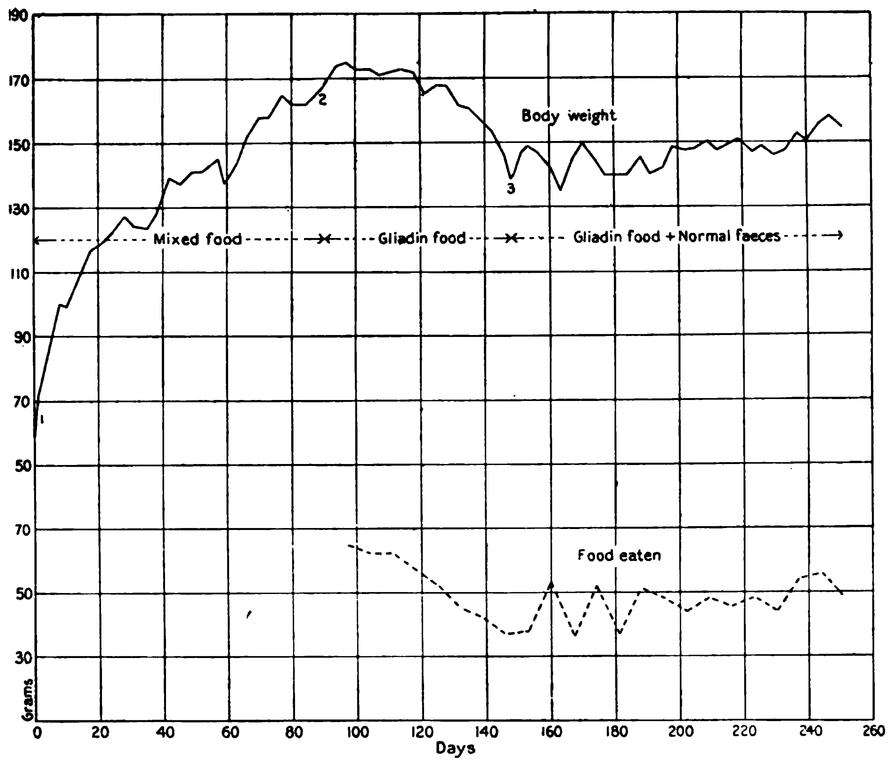


CHART CII.

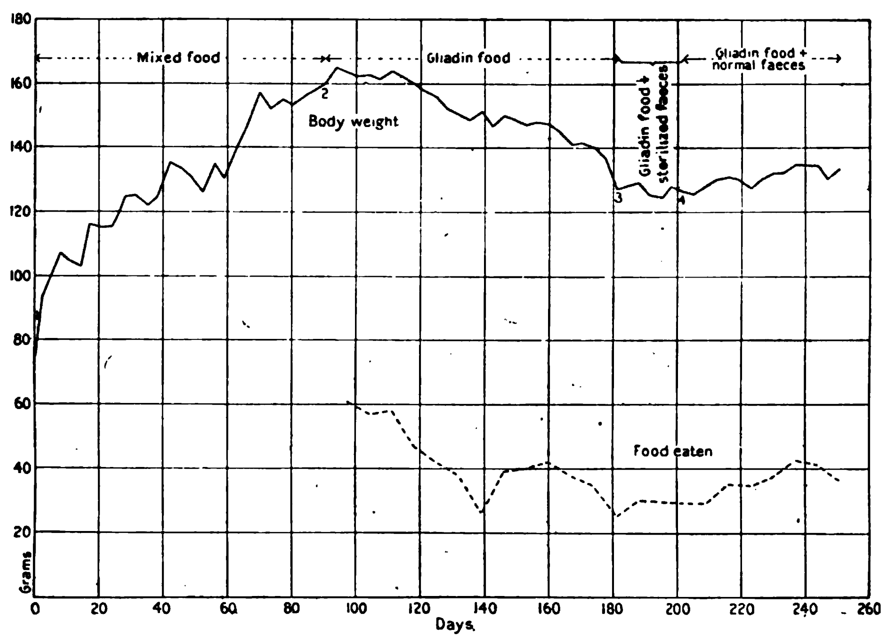


CHART CIII.

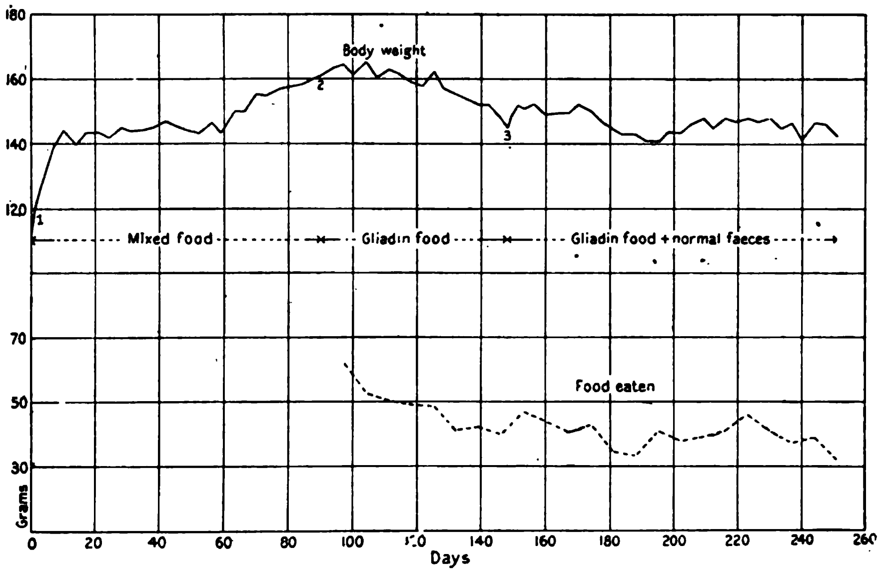


Chart CIII (rat 130, female). The animal, well nourished on a mixed diet during period 1, failed to maintain its body-weight on a diet in which gliadin was the sole protein (period 2), until fæces were added in period 3. The diet consisted of mixed food during period 1; for periods 2 and 3 it was as shown.

	p. ct.	
Gliadin (from wheat)	18.0	....
Starch	29.5 to	34.5
Sugar	15.0	17.0
Agar	5.0	....
Salt mixture I	2.5	....
Lard	25.0	30.0

CHART CIV.

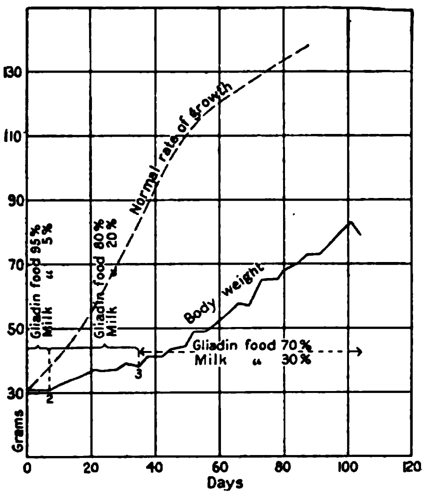


CHART CV.

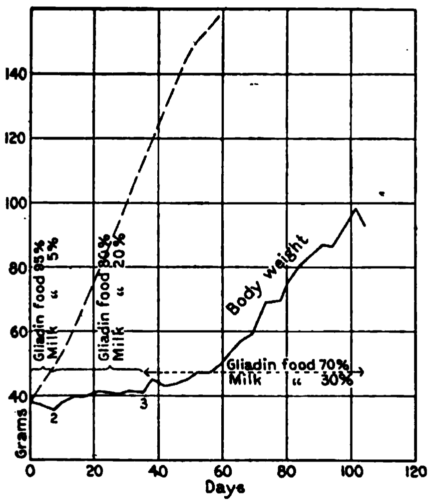


CHART CVI.

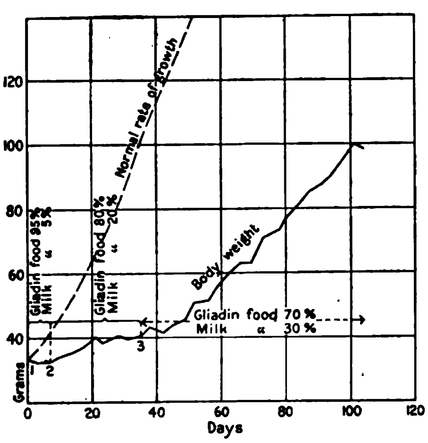
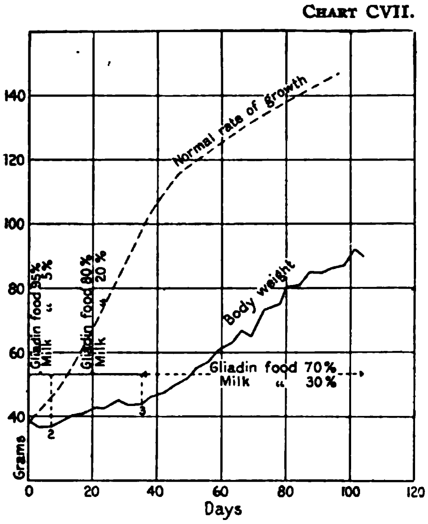


CHART CVII.



Charts CIV (rat 234, female), CV (rat 228, female), CVI (rat 235, male), and CVII (rat 227, male) show the effect of successively larger additions of milk-paste to gliadin food mixture which has been shown in other experiments to be inadequate for the purposes of growth. Note the more rapid growth as the content of milk is increased. The diet consisted of—

Constituents.	Per. 1.	Per. 2.	Per. 3.
	p. ct.	*p. ct.*	*p. ct.*
*Gliadin food.....	95	80	70
†Milk food.......	5	20	30

*Gliadin food: gliadin (from wheat) 18.0; starch, 29.5 to 32.5; sugar, 17.0; agar, 5.0: salt mixture I, 2.5; lard, 25 to 28.

†Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3.

CHART CVIII.

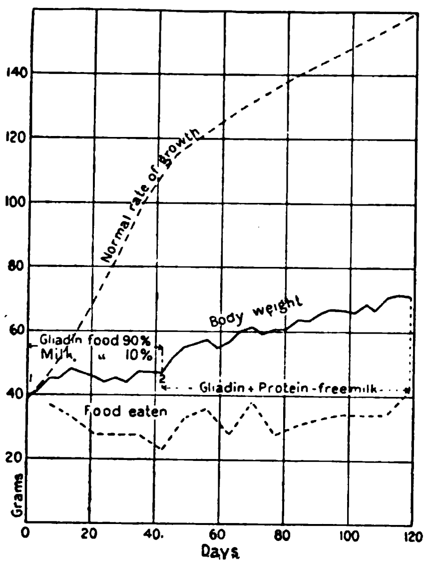


CHART CIX.

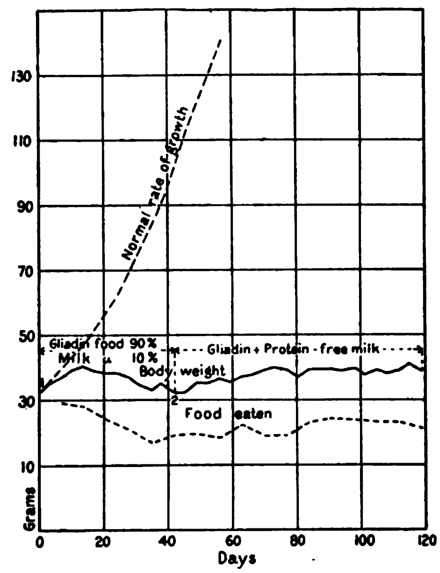


CHART CX.

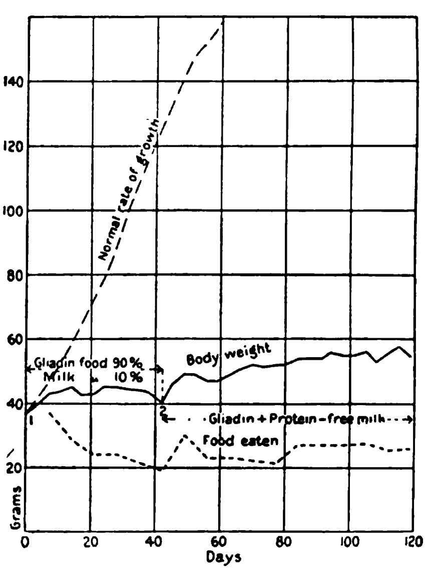
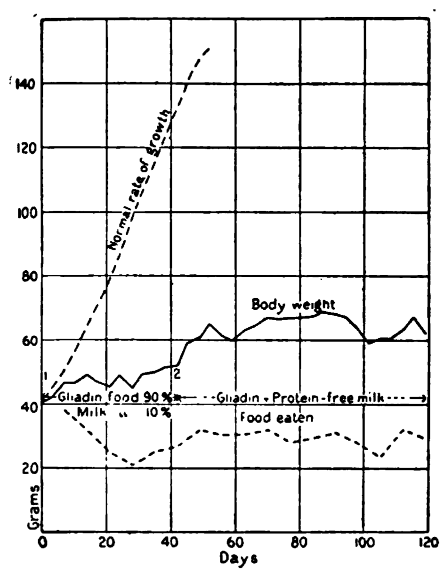


CHART CXI.



Charts CVIII (rat 214, female), CIX (rat 219, male), CX (rat 220, male), and CXI (rat 213, male) show the failure to induce more than slight growth when gliadin forms the sole protein of the dietary, even under conditions in which most other proteins have been found effective. That the failure to grow is not due to insufficient food intake is evident. The character of the diets is given in the table below.

Period 1:	p. ct.
Gliadin food: gliadin (from wheat), 18.0; starch, 32.5; sugar, 17.0; agar, 5.0; salt mixture I, 2.5; lard, 25.0	90
Milk food: Trumilk, 60.0; starch, 15.7; salt mixture I, 1.0; lard, 23.3	10

Period 2:	p. ct.
Gliadin (from wheat)	18.0
Protein-free milk	28.2
Starch	20.8
Agar	5.0
Lard	28.0

CHART CXII.

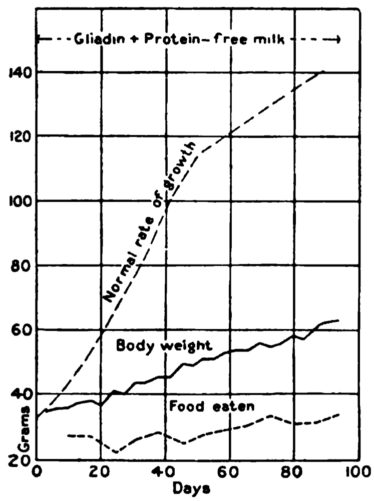


CHART CXIV.

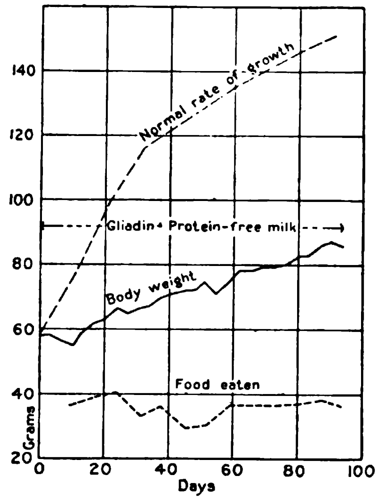
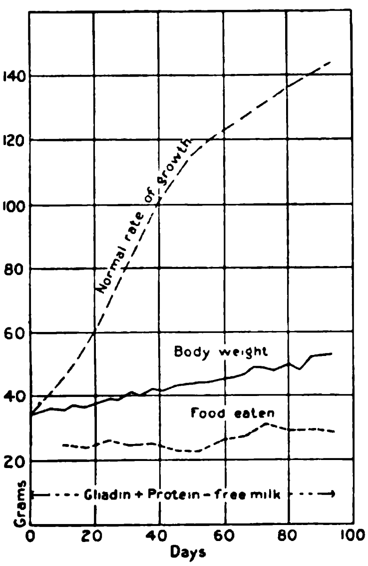


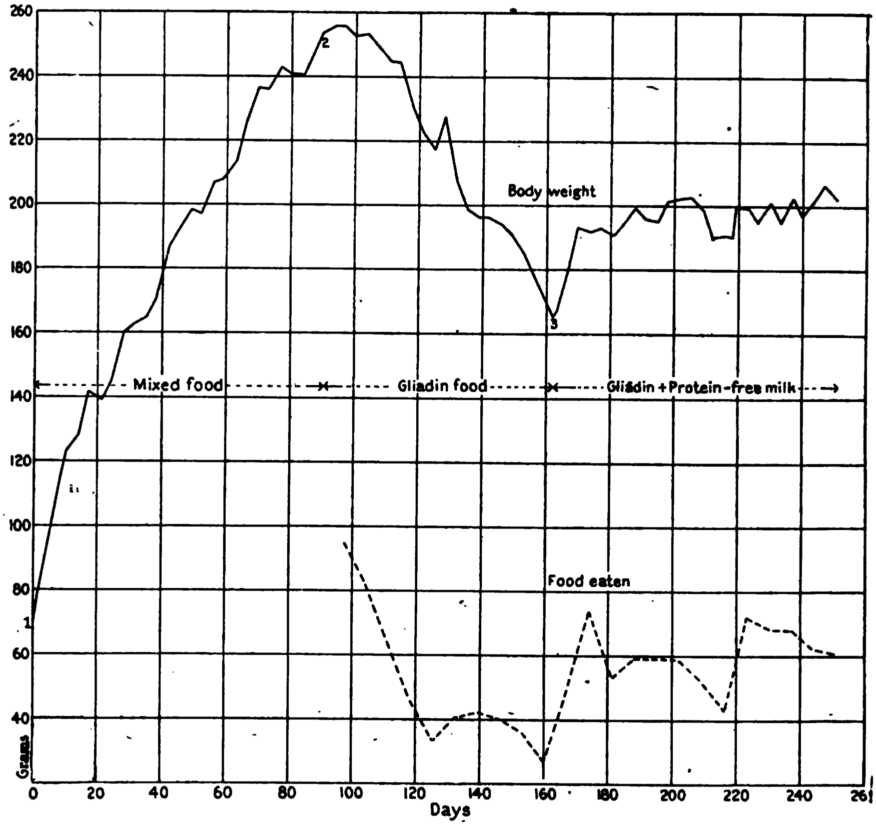
CHART CXIII.



Charts CXII (rat 249, female), CXIII (rat 240, female), and CXIV (rat 254, female) show the failure of the animals to grow normally on a diet containing protein-free milk and gliadin as the sole protein. It will be noted that these animals ate well and that the maintenance was better than with similar gliadin mixtures which contained no protein-free milk. The composition of the food was as follows:

	p. ct.
Gliadin (from wheat).......	18.0
Protein-free milk...........	28.2
Starch.....................	20.8
Agar.......................	5.0
Lard.......................	28.0

CHART CXV.



Charts CXV (rat 144, male) and CXVI (rat 134, female) show the failure of animals previously well nourished on a mixed diet (period 1), to be maintained on a diet in which gliadin formed the sole protein (period 2), until protein-free milk was added to the food (period 3). The food during period 1 was mixed. During periods 2 and 3 it was as shown herewith.

Constituents.	Per. 2.	Per. 3.
	p. ct.	*p. ct.*
Gliadin (from wheat)	18.0	18.0
Protein-free milk	0.0	28.2
Starch	29.5 to 34.5	20.8
Sugar	15.0 17.0	0.0
Agar	5.0	5.0
Salt mixture I	2.5	....
Lard	25.0 30.0	28.0

Chart CXVII (rat 129, female) shows the maintenance in period 2 on a diet containing protein-free milk and gliadin as the sole protein. Note that the animal did not decline like those fed on gliadin without protein-free milk. The preliminary period 1 on a mixed diet, during which the animal was twice pregnant, is introduced to show the excellent previous nutritive condition of the rat. The composition of the food for period 1 was mixed; for period 2 it was as shown herewith.

	p. ct.
Gliadin (from wheat)	18.0
Protein-free milk	28.2
Starch	20.8
Agar	5.0
Lard	28.0

CHART CXVI.

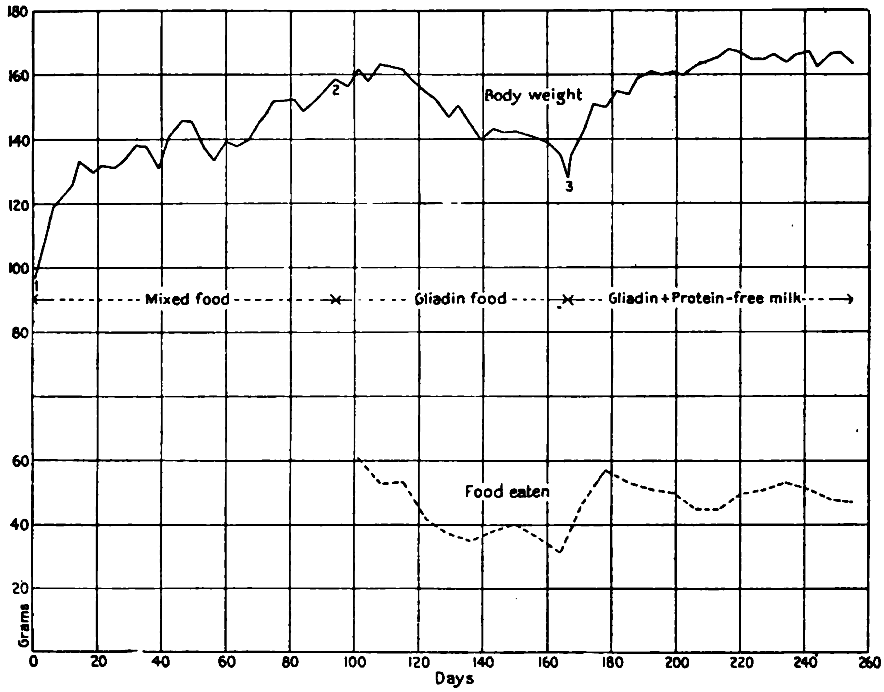


CHART CXVII.

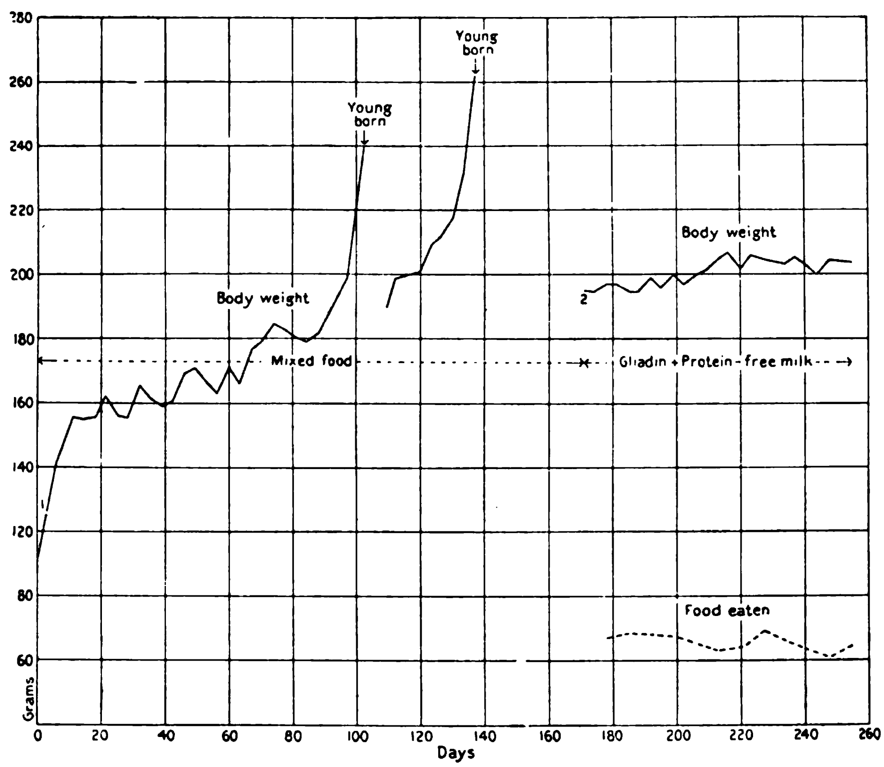


CHART CXVIII.

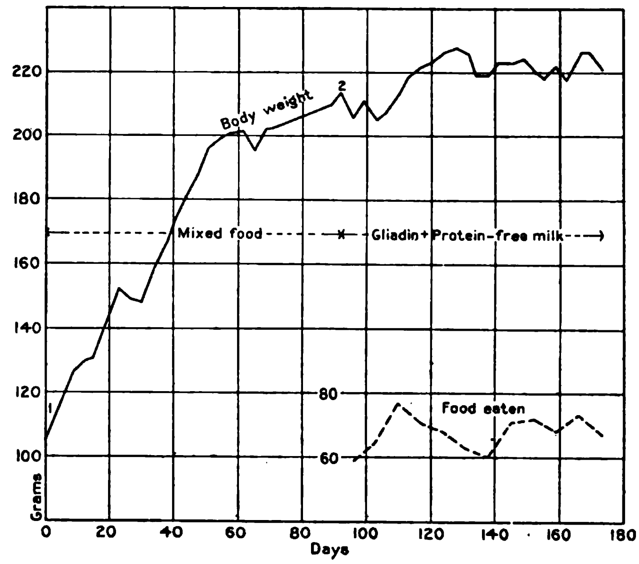
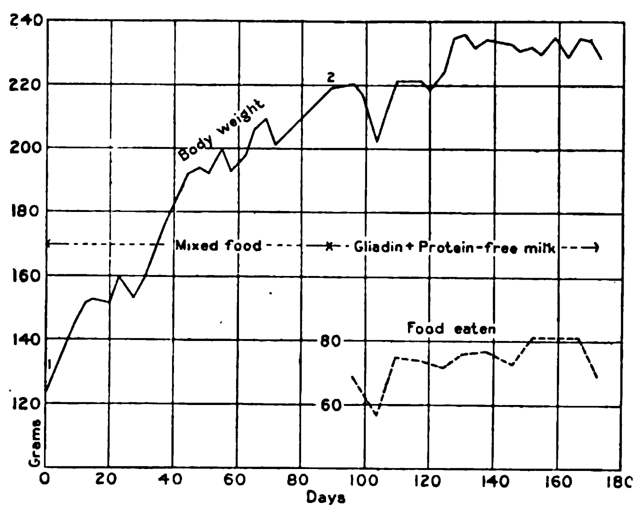


CHART CXIX.



Charts CXVIII (rat 167, male) and CXIX (rat 168, male) show maintenance in period 2 on a diet containing protein-free milk and gliadin as the sole protein. The animals did not decline like those fed on gliadin without protein-free milk. Note their abundant food intake. The preliminary period is introduced to show the excellent previous nutritive condition of the rats. The composition of the food was mixed during period 1; for period 2 it was as shown herewith.

	p. ct.
Gliadin (from wheat)...	18.0
Protein-free milk.......	28.2
Starch..................	20.8
Agar....................	5.0
Lard....................	28.0

CHART CXX.

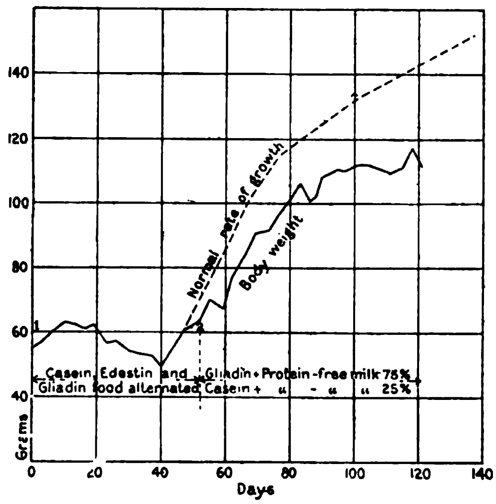
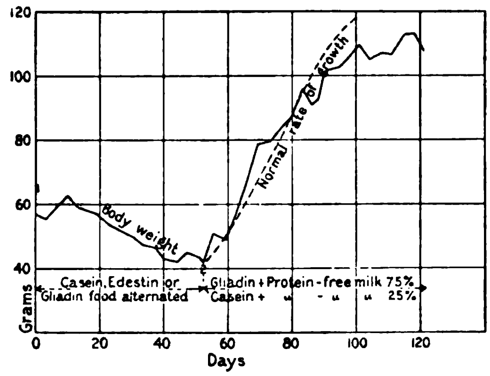


CHART CXXI.



Charts CXX (rat 208, female) and CXXI (rat 206, female) show, in period 1, failure to grow on the diet indicated below; and, in period 2, nearly normal growth on a diet containing protein-free milk in which one-quarter of the gliadin, previously found inadequate to induce growth, was replaced by casein. Note the small quantity of casein which suffices to promote growth instead of standstill. This emphasizes the different nutritive value of casein and gliadin. The diets consisted of—

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein or Edestin or Gliadin	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0)	75
Starch	32.5	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)	25
Sugar	17.0		
Agar	5.0		
Salt mixture I	2.5		
Lard	25.0		

CHART CXXII.

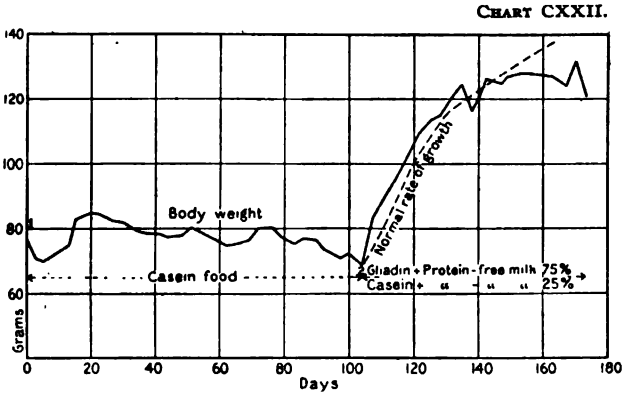


Chart CXXII (rat 179, female). Period 1 shows maintenance without growth on a diet containing salt mixture I (no protein-free milk) and casein as the sole protein. This should be contrasted with numerous similar experiments in which the inorganic constituents of the diet were present in the

form of protein-free milk. Period 2 shows the influence of the substitution by casein of one-fourth of the gliadin in a dietary repeatedly shown to suffice for maintenance but not for growth. This emphasizes the different nutritive value of casein and gliadin. The composition of the diets was as shown below.

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0)	75
Starch	32.5		
Sugar	17.0 to 20.0		
Agar	5.0	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)	25
Salt mixture I	2.5		
Lard	22.0 to 25.0		

CHART CXXIII.

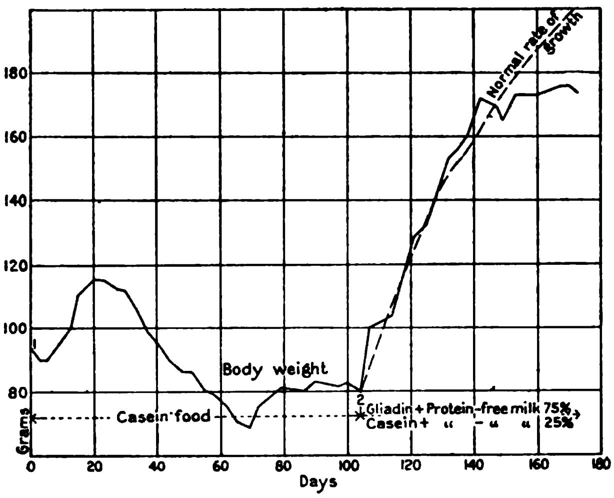


Chart CXXIII (rat 173, male). Period 1 shows imperfect maintenance without growth on a diet containing salt mixture I (no protein-free milk) and casein as the sole protein. This should be contrasted with numerous similar experiments in which the inorganic constituents of the diet were present in the form of protein-free milk. Period 2 shows the influence of the substitution by casein of one-fourth of the gliadin in a dietary repeatedly shown to suffice for maintenance but not for growth. This emphasizes the different nutritive value of casein and gliadin. The composition of the diets was—

Constituents.	Per. 1.	Constituents.	Per. 2.
	p. ct.		*p. ct.*
Casein	18.0	Gliadin food (gliadin (from wheat), 18.0; protein-free milk, 28.2; starch, 20.8; agar, 5.0; lard, 28.0.)	75
Starch	32.5		
Sugar	17.0 to 20.0		
Agar	5.0	Casein food (casein, 18.0; protein-free milk, 28.2; starch, 23.8; agar, 5.0; lard, 25.0)	25
Salt mixture I	2.5		
Lard	22.0 25.0		

CHART CXXIV.

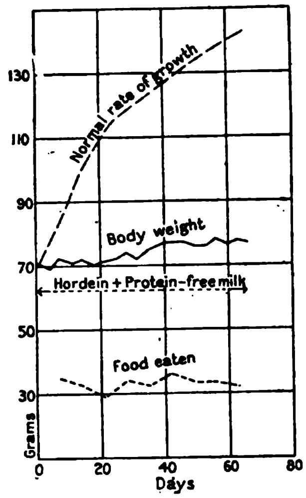
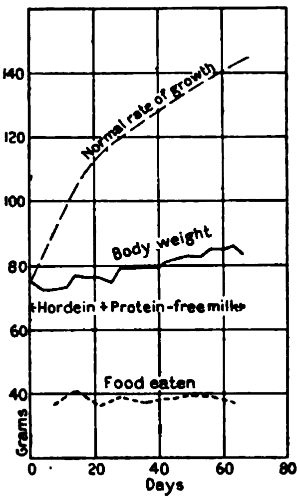


CHART CXXV.



Charts CXXIV (rat 256, female) and CXXV (rat 255, female) show maintenance without growth of medium-sized rats on a diet of protein-free milk and hordein, from barley, as the sole protein. Note the undiminished appetite during course of experiment. Precisely similar mixtures containing other single proteins have sufficed to induce growth. This experiment demonstrates the different nutritive value of hordein and most other proteins and its resemblance in this respect to the chemically similar protein gliadin. This is a marked instance of the relation of the chemical constitution of the protein to nutrition. The composition of the food was as shown herewith.

	p. ct.	
Hordein	18.0	....
Protein-free milk	28.2	....
Starch	16.8 to	18.8
Agar	5.0	....
Lard	30.0	32.0

CHART CXXVI.

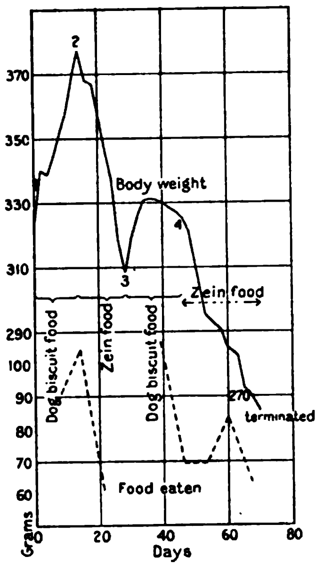
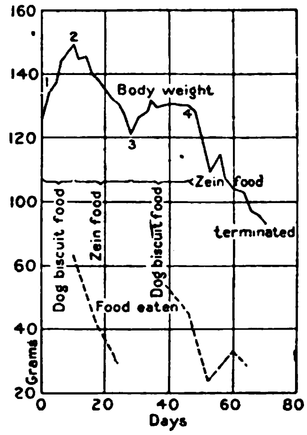


CHART CXXVII.



Charts CXXVI (rat XI) and CXXVII (rat XIV) show the failure of well-nourished animals (see period I) to be maintained on a diet in which zein formed the sole protein. The diet consisted of—

Constituents.	Periods 1 and 3.	Constituents.	Per. 2.	Per. 4.
	p. ct.		p. ct.	p. ct.
Dog biscuit......	58.33	Zein..........	16.89	10.77
Lard............	41.66	Starch.......	10.14	23.70
		Sugar........	8.78	21.54
		Salt mixture..	3.38	2.15
		Agar.........	10.14	5.17
		Lard.........	50.67	36.63

CHART CXXVIII.

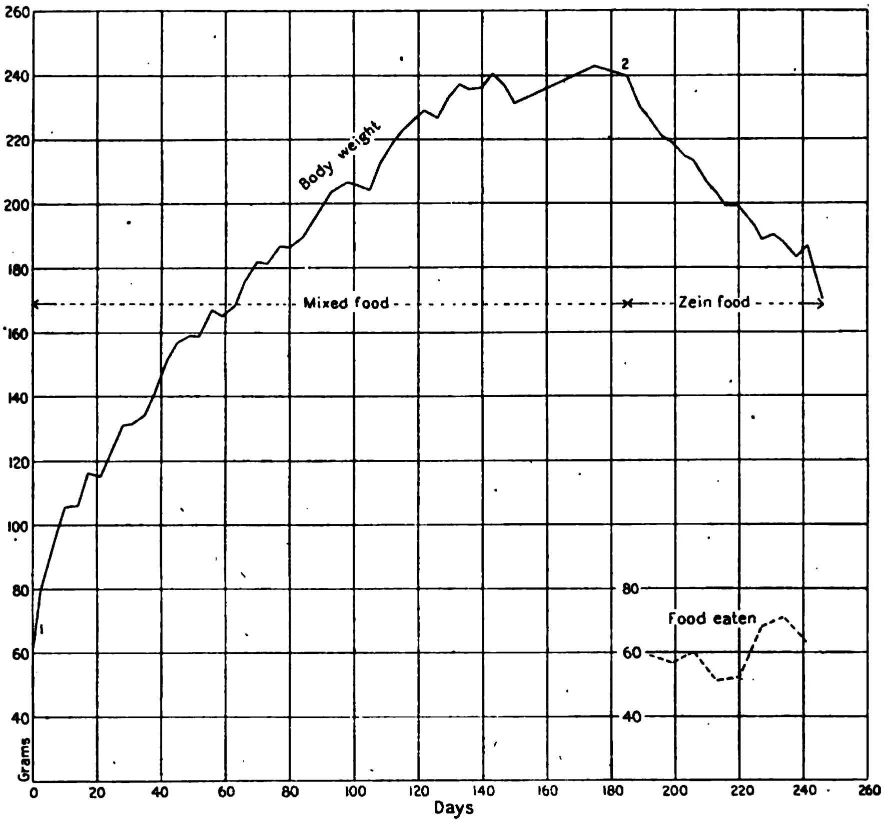


Chart CXXVIII (rat 146, male) shows the failure of a well-nourished rat (see period 1) to be maintained on a diet containing protein-free milk and zein as the sole protein. It should be noted that precisely similar mixtures in which zein was replaced by any of the other proteins studied, sufficed either to induce growth or at least to maintain body-weight for an equally long period. Attention is directed to the continued fall in weight despite the large food intake. The composition of the food was mixed for period 1; for period 2 it was as shown herewith.

Period 2.	*p. ct.*
Zein	18.0
Protein-free milk	28.2
Starch	23.8
Agar	5.0
Lard	25.0

CHART CXXIX.

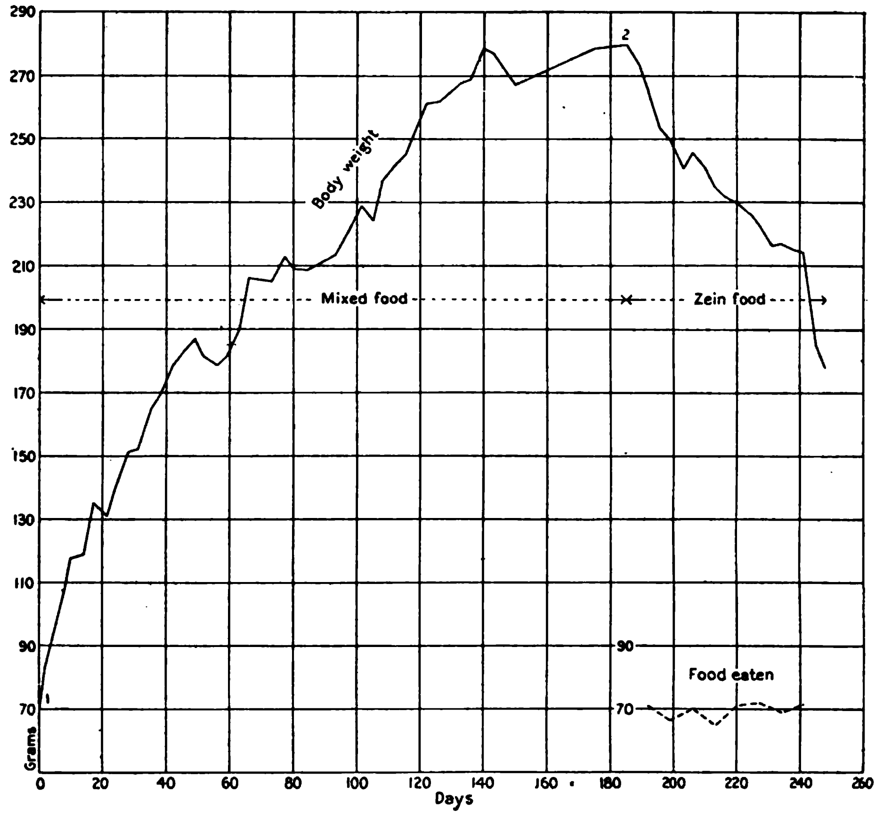


Chart CXXIX (rat 157, male) shows the failure of a well nourished rat (see period 1), to be maintained on a diet containing protein-free milk and zein as the sole protein. It should be noted that precisely similar mixtures in which zein was replaced by any of the other proteins studied, sufficed either to induce growth or at least to maintain body-weight for an equally long period. Attention is directed to the continued fall in weight despite the large food intake. The composition of the food was mixed for period 1; for period 2 it was as shown herewith.

Period 2.	*p. ct.*
Zein..............	18.0
Protein-free milk.	28.2
Starch...........	23.8
Agar.............	5.0
Lard.............	25.0

NEW HAVEN, CONNECTICUT, U. S. A.,
JULY 1, 1911.